DIET AND MOVEMENT OF DEPREDATING MALE SPERM WHALES (*Physeter macrocephalus*) IN THE GULF OF ALASKA

By

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Abstract

Sperm whales (*Physeter macrocephalus*) remove fish from commercial fishing gear in high latitude foraging grounds. This behavior, known as depredation, occurs in the Gulf of Alaska (GOA) sablefish longline fishery and has increased in frequency and severity since the mid-1990s. Sperm whale foraging ecology and movements in the GOA are poorly understood but are important considerations to how depredation impacts fishery resources and whale behavior. The goals of this dissertation were to use stable isotope analysis to evaluate trophic connections between sperm whales and their prey, estimate the proportional contribution of various prey items to sperm whale diets, and use satellite tag data to evaluate movement and diving behavior of sperm whales in the GOA. Understanding isotopic variability in cetacean skin is important to evaluating dietary information from this tissue; thus, in chapter 1, I first analyzed the stable isotope ratios among layers of cetacean skin to determine how much variability there was within and across layers of cetacean skin. Results showed horizontal layers of cetacean skin to be significantly different isotopically, suggesting evidence of a dietary time series in layers of cetacean skin, where the innermost skin layer represents the most recent diet. These results were used in my second chapter to isolate the most recent diet of sperm whales from the inner layer of skin, and then to estimate proportional contributions of different prey to sperm whale diets. Results showed that the sperm whales sampled prefer sablefish, dogfish, skates, and rockfish, and that the proportional contribution of sablefish to sperm whale diets has increased over the past 15 years as depredation has increased in severity. Chapter three presented an analysis of twenty-nine satellite tags placed on depredating sperm whales in the GOA between 2007 and 2016 to explore movement and diving behavior and how these behaviors may be linked to prey preferences found in chapter 2. Tagged sperm whales in the GOA preferred the continental slope habitat and made long migrations along the slope toward Mexico and the Gulf of California, speeding up and switching behaviors from foraging to transiting when they left the GOA. Dive depths and durations exhibited individual variability and were significantly correlated to light levels, lunar cycles, sablefish fishery catch-per-unit-effort, and seafloor depth. Results suggest diving behavior tracks that of primary groundfish prey items, and dive depths become shallower in areas of high sablefish densities, as inferred from fishery catches, potentially reflecting depredation behavior. Together these results provide a much-improved understanding of the

impact of depredation on sperm whale dietary preference, and show insights into the importance of the GOA as a foraging ground for endangered sperm whales.

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General Introduction

Sperm whales (*Physeter macrocephalus*) are the largest toothed whale on the planet, a deep diving species that inhabits all of the world's major oceans (Whitehead, 2003). They are a difficult species to access and study due to their long deep dives and their offshore habitat (Rice, 1989; Whitehead, 2003). Females are thought to primarily inhabit warmer equatorial waters, while males migrate to higher latitude foraging grounds after reaching age 10 and become more solitary (Whitehead, 2003). These high-latitude foraging grounds are often in remote areas, where males can be widespread, adding a degree of additional difficulty to studying this species.

Sperm whales increasingly interact with commercial fisheries worldwide, a behavior that is seen primarily in males in their high-latitude foraging grounds. In the Southern Ocean, they remove Patagonian toothfish from commercial longline fishing gear off of South Georgia Island, as well as the Crozet and Kerguelen Islands (Ashford *et al.*, 1996; Purves *et al.*, 2004; Tixier *et al.*, 2010; Guinet *et al.*, 2015). Australian and Chilean toothfish fisheries also experience depredation by sperm whales (Moreno *et al.*, 2008; Hamer *et al.*, 2012, 2015). In Norway, sperm whales have recently begun removing halibut from demersal longline fishing gear (Tiu Similä, unpublished data). In the Gulf of Alaska (GOA), sperm whales remove sablefish from demersal longline fishing gear in an interaction that was first documented in the mid 1990's and has increased in severity since then (Hill *et al.*, 1999; Sigler *et al.*, 2008; Straley *et al.*, 2015; Hanselman *et al.*, 2018a).

In 2003, the Southeast Alaska Sperm Whale Avoidance Project (SEASWAP) was first funded with the aim of better understanding and minimizing the effects of depredation in the GOA. SEASWAP is unique in that it was initiated by fishermen; it is a collaboration between fishermen, scientists, and managers to study depredation and test countermeasures (Straley *et al.*, 2015). Using fishing vessels as a platform for research, SEASWAP has identified an acoustic cue alerting whales to fishing activity (Thode *et al.*, 2007), documented the spread of the behavior as a potential example of social transmission (Schakner *et al.*, 2014), engaged in acoustic research to better understand whale behavior around longline vessels (Mathias *et al.*, 2012), and tested a variety of deterrent devices (Thode *et al.*, 2010; O'Connell *et al.*, 2015; Wild *et al.*, 2017). Management concerns and fishermen questions often drive SEASWAP research projects; two of

these areas of interest consist of questions about the diet and movement of sperm whales in the GOA.

Sperm whales worldwide are thought to primarily consume cephalopods, but in some regions they eat a variety of deep-sea fish species (Pike, 1950; Berzin, 1959; Gaskin and Cawthorn, 1967; Kawakami, 1980; Whitehead, 2003). The diet of this top predator is of particular interest to commercial fishermen in the GOA, due to their increasing propensity to engage in depredation behavior. Fishermen and fisheries scientists and managers are keen to better understand how important sablefish were to historic sperm whale diets in the GOA, and how important they are to current diets. Most of what is known about sperm whale diets in the GOA consists of records kept of stomach contents from commercial whaling ships and at whaling stations. Recently, stable isotope analysis has become a minimally invasive method to study diet and trophic connections of marine species. Following the old adage "you are what you eat", the chemical composition of prey species is incorporated into predators' tissues, though with some variability and uncertainty in the diet-tissue incorporation rates (Tieszen *et al.*, 1983; Thomas and Crowther, 2015), the turnover rates of tissues (Hobson and Clark, 1992a; Vander Zanden *et al.*, 2015), and of the trophic enrichment factors (TEFs) between prey and predators (Newsome *et al.*, 2010).

Similar to gaps in our limited understanding of the diet of male sperm whales on their high latitude foraging grounds, the movement of males has been identified as a major knowledge gap (Whitehead, 2003). Preliminary work has shown that some of these animals move from the GOA to Mexico along the continental slope (Straley *et al.*, 2014), but additional research is necessary to better understand both broad-scale and local movements, and how they are influenced by depredation behavior. Movement data can also be used to identify foraging hotspots and shed light on population dynamics to better understand stock structure of these endangered species. Finally, foraging hotspots and movement of marine predators is often driven by prey availability. Analysis of environmental covariates of diving behavior (dive depth and duration) can provide insights into important drivers of foraging behavior and movement.

In the first two chapters of my dissertation, I explore the use of stable isotope ratios in dietary research of large whales. First, in Chapter 1, I explore the variability in stable isotope ratios within the skin of three cetacean species with very different diets, the humpback whale, the fin whale, and the sperm whale. Each of these species has markedly different dietary preferences:

humpback whales feed on a variety of schooling fish (e.g. herring, capelin, sand lance) and krill, that have very different known stable isotope ratios (Witteveen *et al.*, 2012; Wright *et al.*, 2015; Witteveen and Wynne, 2016); fin whales have a fairly homogenous diet of euphausiids (Borrell *et al.*, 2012; Witteveen and Wynne, 2016); and sperm whales are generalist predators feeding on a variety of deep-sea fishes and squid, which are themselves poorly understood isotopically. By cutting each skin sample into three horizontal layers and three vertical cores, I evaluate the use of different portions of skin to reflect a dietary time series of stable isotope ratios. I used the findings from Chapter 1 in Chapter 2 to isolate a portion of the skin representing the most recent diet and exploring the isotopic variability between sperm whales and their prey in the GOA. For this chapter, I sampled both sperm whales and known prey in the GOA and built a catalog of the first published stable isotope ratios of many of these species in this region. I then used isotopic mixing models to assign proportional contributions of prey items to sperm whale diets. For these models, I first assessed diets from all sperm whale samples combined, and then divided the sperm whale samples into various groupings to compare dietary composition. Three groupings of sperm whales were compared: 1) temporal (across years) - older versus more recently collected sperm whale samples were compared to evaluate changes in dietary composition over the past 15 years; 2) temporal (within season) - early, mid, and late summer samples were compared to explore seasonal variability in dietary composition; and 3) behavioral - sperm whales seen more frequently depredating versus more non-frequent depredators were compared to address variability in diets of whales. These groupings allowed me to evaluate how sablefish contribution to sperm whale diets has increased over the 15-year time span of our biopsy samples, how sablefish contribution to diets changes throughout the summer season between May and September, and how diets of more frequently sighted sperm whales differ from that of whales sighted less frequently around fishing vessels.

The third chapter of my dissertation examines satellite tag data from SEASWAP's archives collected since 2007, building upon an initial analysis completed by Straley et al. (2014). General patterns of movement from tags are analyzed, and dive behavior data available from half of the tags are also summarized. I use state-space models (SSMs) to estimate behavioral states and true locations from the stochastic movement processes of sperm whale tag data (Jonsen *et al.*, 2013). Finally, I use generalized additive models (GAMs) to explore the relationship between diving behavior (maximum dive depth and dive duration) and

environmental covariates such as seafloor depth, day of the year, lunar cycle, diel cycle, slope, and sablefish fishery catch-per-unit-effort (CPUE). Together, this work will add to our knowledge of the diet and movement of sperm whales in the GOA and provide insights the drivers of depredation that are useful for both fishermen and fisheries managers.

Chapter 1 Evidence for dietary time series in layers of cetacean skin using stable carbon and nitrogen isotope ratios[1]

1.1 Abstract

Stable isotope analysis integrates diet information over a time period specific to the type of tissue sampled. For metabolically active skin of free-ranging cetaceans, cells are generated at the basal layer of the skin and migrate outward until they eventually slough off, suggesting potential for a dietary time series. Skin samples from cetaceans were analyzed using a continuous-flow elemental analyzer isotope ratio mass spectrometer (EA-IRMS). We used ANOVAs to compare the variability of $\delta^{13}C$ and $\delta^{15}N$ values within and among layers and columns ("cores") of the skin of a fin, humpback, and sperm whale. We then used mixed-effects models to analyze isotopic variability among layers of 28 sperm whale skin samples, over the course of a season and among years. We found layer to be a significant predictor of $\delta^{13}C$ values in the sperm whale's skin, and $\delta^{15}N$ values the humpback whale's skin. There was no evidence for significant differences in $\delta^{15}N$ or $\delta^{13}C$ values among cores for any species. Mixed effects models selected layer and day of the year as significant predictors of $\delta^{13}C$ and $\delta^{15}N$ values in sperm whale skin across individuals sampled during the summer months in the Gulf of Alaska. These results suggest that skin samples from cetaceans may be subsampled to reflect diet during narrower time periods; specifically, different layers of skin may contain a dietary time series. These results underscore the importance of selecting an appropriate portion of skin to analyze based on the species and objectives of the study.

1.2 Introduction

Understanding changes in an animal's diet and the temporal scale over which those changes occur can give valuable insight into habitat preferences, migration patterns, and potential for interactions with humans. Stable isotopes have become an increasingly important method for the study of marine mammal diets, trophic niche, movement patterns, physiology, historical

[1] Wild, L.A., Chenoweth, E.M., Mueter, F.J., and Straley, J.M. 2018. Evidence for dietary time series in layers of cetacean skin using stable carbon and nitrogen isotope ratios. Rapid Commun Mass Spectrom. 32(16):1425-38.

ecosystem parameters, and other ecological studies [1–7]. Stable carbon isotope ratios ($\delta^{13}C$) are used as a proxy for determining foraging habitat because, in marine systems, pelagic and offshore areas tend to have lower carbon isotope values than benthic and near-shore areas [8–11]. Stable nitrogen isotope ratios ($\delta^{15}N$) are an indicator of trophic position due to metabolic fractionation with each trophic level [12–18]. Specifically $\delta^{15}N$ values increase 2-4‰ per trophic level in marine mammals [4,19–22]. For marine mammal species, the majority of analyses over recent years have focused on single-species niche breadth [23–27], comparative diet studies [1,3,28–31], and mixing models to determine diet composition [29,32]. Unlike samples from stomach lavage or feces, stable isotope analysis of consumer tissues integrates diet over a longer time period than a single meal or day, and samples are more accessible from free-ranging cetaceans [33–35]. However, key metabolic parameters allowing for precise interpretation of the temporal resolution of stable isotope results are often lacking, especially for metabolically active tissues. Additionally, the variability of stable isotope ratios from different samples within a tissue is not well-established, though existing studies indicate that replicate samples of muscle tissue from individual fish and zooplankton produced variability within a single individual of <0.2‰, and a recent study with common and striped dolphins showed there were no significant differences among stable isotope ratios across various locations on the skin [36–39]. This variability is essential context to appropriately interpret differences in stable isotope ratios observed among tissues or individuals.

To properly interpret stable isotope ratios of animal tissue, it is essential to account for the temporal dynamics of isotopic integration. Two aspects of integration are important to consider: diet-tissue incorporation rate and tissue turnover rate. Incorporation rate refers to the amount of time between prey ingestion and when the isotopic signature of that prey item enters the tissue being analyzed [40,41]. This rate can be estimated in captive cetaceans through controlled feeding experiments, but is not well understood in free-ranging cetaceans, and depends on biochemical processes such as thermoregulation type, growth rate, reproductive status, and environmental stressors [21,22,33,40,42]. Animal tissues can be categorized as metabolically active or inert. Metabolically active tissues have different isotopic incorporation rates, while interpretation of isotope patterns in metabolically inert tissues depends on tissue growth rates. Turnover rate refers to the rate of replacement of the tissue [43,44]. Turnover rates are more widely studied in marine mammals, and can be short in metabolically active tissues such as liver or blood, or much

longer in inert tissues such as whiskers, baleen, bone, and hair. Differences in turnover rate is one of the causes of variability in stable isotope signatures within and between tissues [33,45]. Turnover rates in stable isotope analysis are often discussed in terms of half-life of the tissue, or the time at which half of the tissue has been replaced [33,46,47]. Inert tissues such as claws, baleen, and hair often preserve a dietary time series for individual marine mammals [7,23,27,30,48,49]. In these tissues, diet-tissue incorporation occurs toward the basal layer of the tissue where new cells are generated, and turnover rate is related to the growth rate of the tissue and thickness of the sample, with shorter turnover rates for thinner sections [21,40,41,46]. Controlled feeding experiments of captive animals have been used to study turnover rates for skin of smaller cetacean species (Table 1.1). Such experiments can only be performed on captive animals, excluding direct measure for baleen whales.

Skin tissue (epidermis) is often used to study diet in free-ranging cetaceans because its collection is minimally-invasive [2,29,34,50,51]. Current stable isotope analysis of cetacean skin treats the skin as a homogenous tissue in terms of dietary information and uses a variety of methods to select the portion of skin used in analysis. Most common are analyses using the outer layer of skin as it sloughs off [52], homogenizing a small piece of skin cut from an undefined portion of a biopsy sample [2,35,53], extracting a section of skin from a stranded carcass [25,54], or a combination of these sampling methods [1,55].

Cetacean skin is divided into layers based on cellular structure, often referred to as the stratum basale/germinativum (inner), stratum spinosum (intermediate), and stratum externum (outer) [56,57]. Cells in the stratum basale tend to be primarily large, tall epithelial cells that are well-developed with many mitochondria, ribosomes, desmosomes, and lipid droplets. Cells in the stratum spinosum tend to have fewer mitochondria and ribosomes, but the same number of lipid droplets and desmosomes. This layer shows cells that are rounded or oval in shape, and they become flattened as they migrate out towards the stratum externum, where cells show keratinization and lipid droplets are densely packed. In the stratum externum the nucleus is often remnant, and sometimes completely intact, indicating keratinization is not complete [56,57]. Because epidermal cells are generated at the basal layer of skin (stratum basale) and migrate outward [58,59], there is the potential for a dietary time series to exist within cetacean skin. The

basal layer of cetacean skin, adjacent to the dermis, is textured rather than flat [60]. Small dermal papillae extend between a quarter to half-way up into the epidermis from the dermis [58–60]. Hicks *et al.* [58] labeled sub-epidermal cells in bottlenose dolphins to examine the rate of skin turnover. Labeled cells migrated first laterally out of the intruding papillae, and then vertically toward the outer layer of skin, before flaking off as the animal sloughed its skin (Figure 3 in [58]). Labeled cells entering the skin from the tip of the dermal papillae in the stratum spinosum, were able to move directly outward toward the surface in the stratum externum, with no lateral movement. Therefore, new cells may be found from the stratum basale to the furthest intrusion of the papillae in the stratum spinosum (Figure 3 in [58]). This, combined with information on cellular differences between strata, suggests that epidermal cells get older on average in tissue further from the dermal-epidermal interface. Busquets-Vass *et al.*[41] hypothesized that due to this stratification within the skin, skin might contain a dietary time series. In stable isotope analysis of blue whale (*Balaenoptera musculus*) skin, Busquets-Vass *et al.* found $\delta^{15}N$ values to vary significantly between the inner (stratum basale) and outer (stratum externum) layers of skin in the California Current System, which corresponded temporally to differences in $\delta^{15}N$ values of prey in two seasonal foraging grounds [41].

Among cetaceans in the Gulf of Alaska (GOA), there is a wide range of variability in diets, which vary considerably among individuals in some species, but are more homogenous in other species. Fin whales (*Balaenoptera physalus*) forage in both near-shore and offshore waters in the GOA. Fin whales worldwide have a fairly homogenous lower-trophic diet of euphausiid krill (*Euphausiacea*) and occasionally small amounts of small schooling fish and copepods [19,61–65]. Humpback whales (*Megaptera novaeangliae*) are generalist predators in coastal Southeast Alaskan waters, feeding on a varied diet of krill [66,67], herring [68–70] and other small schooling fish [71], that have a broad range of isotopic values [4,29]. Sperm whales (*Physeter macrocephalus*) are the largest of the toothed whales, and are deep-diving offshore predators [72]. While the current diet of sperm whales in the GOA is poorly understood, stomach content data from historical whaling shows that they feed on a variety of deep-sea fishes and squid [61,73–75]. They are also known to remove sablefish (*Anoplopoma fimbria*) and halibut (*Hippoglossus stenolepis*) from commercial longline fishing gear in the GOA [76–79].

In this analysis, we aim to determine if layers of cetacean skin exhibit isotopically distinct signatures, which may be attributed to temporal shifts in diet. To address this objective we designed a pilot study consisting of two experiments. In the first experiment we tested for differences in $\delta^{13}C$ and $\delta^{15}N$ values both within and among layers of skin of three individual cetaceans: a stranded fin whale, a stranded humpback whale, and a free-ranging sperm whale. We hypothesized that subsamples from within the same layer of skin would be more isotopically similar than subsamples taken from different layers within the same full-thickness vertical core of the sample. More specifically we hypothesized that layer would be a significant predictor of one or both stable isotope ratios in species with variable diets, while core would never be a significant predictor. This hypothesis rests on the assumptions that the cells within the same layer of skin were deposited closer to the same time on average than cells in the same core, and that the animal had undergone a detectable diet shift within the turnover time of the full skin thickness. For the humpback whale and sperm whale, two generalist foragers with diverse and seasonal diets, this was likely the case [61,66–71,73–75]. For the fin whale, with a more homogenous diet, we expected there to be lower variability overall among layers [19,61–65]. A second experiment measured the average differences among layers of a larger number of sperm whale skin samples, and tested if the differences between specific layers were consistent across individuals. We hypothesized that we would again find higher variability among layers in each sperm whale than would be expected from replicate samples within a single layer, but that there would not be consistent differences in stable isotope ratios among layers of skin across individual sperm whales. This hypothesis was based on the assumption that the sperm whales sampled might have individual differences in the timing and direction of diet shifts, timing of seasonal migrations, and timing of seasonal isotopic trends in the baseline of the food web relative to the sampling time.

1.3 Methods

1.3.1 Sample acquisition: Experiment 1

Skin samples were acquired from a dead stranded fin whale, a dead stranded humpback whale, and a biopsy of a free-ranging sperm whale, all in the GOA (Table 1.2). Fin whale skin was acquired from a dead animal that arrived into the port of Seward, Alaska on the bulbous bow of a cruise ship on May 29, 2016 (NMFS Regional stranding ID 2016053). The animal was a sub-

adult male, 50 feet in length, and in good body condition. The portion of skin taken for this analysis came from high on the animal's dorsal flank, below the dorsal fin. Skin was also obtained from a dead humpback whale struck by a cruise ship near Juneau, Alaska on July 27, 2010 (NMFS Regional stranding field ID 2010089). The whale was a non-pregnant female, measured 46 feet in length, and was reported to be in good body condition (blubber thickness 61.3 mm dorsolateral to dorsal fin). During the necropsy, scientists removed a section of skin and blubber from the dorsal surface approximately 10 cm by 7.5 cm, which was stored at -80° C. Skin was subsampled from this section, and measured 7 mm thickness.

Biopsy samples were collected from free-ranging sperm whales between 2003 and 2017 using a Barnett crossbow (150 lb draw weight), with bolts equipped with stainless steel tips measuring 40 mm in length and 7 mm in diameter, with a float for retrieval [50,51]. Biopsy samples collect both skin and blubber, with skin being subsampled for this analysis. A single sample, collected from a male sperm whale in September 2015, was chosen for analysis of experiment 1 due to its large size, suitable for subsampling. The skin from the sperm whale sample measured 6 mm depth, with a diameter of 7 mm.

For stable isotope analysis, we subsampled a small 1-cm^2 section of both the fin and humpback whale skin (full-depth), and the full skin sample of the sperm whale biopsy into 10 pieces (Figure 1.1). Each sample was cut into a 9 x 9 grid of three subsamples per row (laterally along sample), and three per column (vertically through depth of sample), and a tenth fully homogenized subsample (Figure 1.1). Our sub-sampling method produces multiple samples from the same layer, which allows for measures of variability and tests of significance. The lateral, or horizontal cuts, subset each core into "layers" assumed to correspond roughly to the three stratum of skin mentioned earlier in this paper (Figure 1.1). We termed these layers "outer" referring to the stratum externum, the layer of skin adjacent to the whale's environment (sample #s 1-3), "middle" referring to the intermediate layer of skin or stratum spinosum (sample #s 4 - 6), and "inner" referring to the basal layer of skin or stratum basale (sample #s 7-9). Each layer measured approximately 2 mm thick. The vertical columns of skin were cut transversal to the body, which we refer to as "cores" with each core containing samples 1, 4, and 7; 2, 5, and 8; and 3, 6, and 9 (Figure 1.1). These cuts were also made with each core cut at equal width relative

to the full width of the sample. The final portion of the sample was a transversal cut including all layers, which we refer to as the "full" thickness sample, or sample 10.

1.3.2 Sample acquisition: Experiment 2

Between 2003 and 2017 in the eastern GOA, skin from 28 free-ranging male sperm whales was collected via biopsy in the same manner as the sample from experiment 1 (Table 1.2). These samples were collected as part of the Southeast Alaska Sperm Whale Avoidance Project (SEASWAP, www.seaswap.info), which focuses on sperm whales that interact with longline fishing vessels in the eastern GOA, primarily targeting sablefish [76,78]. From each of the samples a single column of skin was cut into subsamples corresponding to the three skin layers described above: "outer", "middle", and "inner" parallel to the angle of the blubber-skin interface of each sample. All samples used in this experiment consisted of enough skin to cut into three layers each measuring approximately 2.3 mm thick.

1.3.3 Sample processing: Experiments 1 & 2

Lipid content and preservation method are known to affect isotopic composition of tissues [80,81]. All skin was stored prior to analysis at -80° C. Six tissue samples from sperm whale biopsies (experiment 2) were stored in a 20% saturation of dimethyl sulfoxide (DMSO). For both experiments, tissue was weighed, cut into small pieces to increase surface area for drying, and then oven-dried at 60°C for 24 hours. In addition to removing the influence of lipids in bulk tissues, extracting lipids can eliminate the effects of storage in DMSO tissues [35,55,82,83]. However, lipid-extraction can alter $\delta^{15}N$ values of some species [84–86], although this has not been tested for sperm whales. Thus we performed a small proof of concept study in which the 28 samples from experiment 2 were subsampled into two pieces with half analyzed without extracting lipids (NLE) and the other half with lipids extracted (LE). The difference between extracted (LE) and non-extracted (NLE) $\delta^{15}N$ values for each sample were analyzed using a one sample t-test for significance. Lipid-extracted samples did not have significantly different $\delta^{15}N$ values than non-extracted samples (t=-1.25, df=44, p=0.22). Meanwhile $\delta^{13}C$ values were significantly different between LE and NLE samples (t=-8.82, df=44, p<0.01), showing they warranted lipid extraction. Finally, C:N ratios of non-lipid-extracted samples ranged from 3.3 to 4.7, which were high

enough to warrant lipid-extraction or correction [87]. Thus all stable isotope ratios in this study were assessed using lipid-extracted values.

For lipid-extraction, dried tissue was soaked in 2 mL of a 2:1 solution of chloroform-methanol for 20 minutes in an ultrasonic bath to extract lipids [84,88,89]. The lipid-filled solution was removed using disposable glass pipettes. This procedure was repeated three times [84,88,89]. After lipid-extraction, samples were rinsed with deionized water, again dried at 60°C for 12-24 hours, and re-weighed. Samples were then ground into a fine powder and 0.2 to 0.4 mg aliquots were placed into tin capsules that were sent to the Alaska Stable Isotope Facility (ASIF) in Fairbanks, AK for processing. At ASIF stable isotope data for bulk stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios was obtained using continuous-flow isotope ratio mass spectrometry (CFIRMS). This method utilizes a Thermo Scientific Flash 2000 elemental analyzer and Thermo Scientific Conflo IV interfaced with a Thermo Scientific Delta V^{Plus} Mass Spectrometer (Bergen, Germany). Isotope composition was expressed as a ratio relative to an international standard, in delta notation (δ) as parts per thousand (‰):

1) $$\delta X(‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right]$$

where X is ^{13}C or ^{15}N, and R represents the respective abundance ratio of each isotope (e.g., $^{13}C/^{12}C$ or $^{15}N/^{14}N$). Reference standards used were Vienna Pee-Dee Belemnite for carbon and atmospheric N_2 for nitrogen. Analytical precision was ±0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

1.3.4 Statistical analysis: Experiment 1

For experiment 1 we used two-way analyses of variance (ANOVAs) to test for significant differences in the $\delta^{13}C$ or $\delta^{15}N$ values by layer and core (as a control). Layers were defined previously as a categorical independent variable with classes inner, middle, and outer. Cores, also defined previously, were also a categorical independent variable with three classes. Cores were used as a control due to evidence that cetacean skin shows isotopic homogeneity across the body [39]. This experiment aimed to examine the influence of the two different variables (layers & cores) on a continuous dependent variable ($\delta^{13}C$ or $\delta^{15}N$ values). This analysis was performed

separately for each isotope and separately on each of the three species. Models were run using the nine samples from each species (3 layer, by 3 core), omitting the full thickness-samples (#10's), as they do not include layers. Models were compared using Akaike Information Criterion with a correction for low sample size (AICc values) [90]. When significant differences among layers or cores were found, post-hoc tests (Tukey's Honest Significance Differences) were used to determine which layers or cores differed significantly [91].

1.3.5 Statistical analysis: Experiment 2

For experiment 2, the samples, due to smaller tissue sizes from biopsies, were cut into 3 layers from a single lipid-extracted column only. Observed differences in isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) among layers were first examined visually by individual. Mixed effects models were then used to model isotope ratios as a function of layer and day of the year (DOY) as follows:

2) $$y_{ijk} = \propto_j + a_i + a_k + \beta_{1,j} * doy + \beta_{2,j} * doy^2 + \varepsilon_{ijk} ,$$

where y_{ijk} is the isotope ratio ($\delta^{13}C$, $\delta^{15}N$) for individual whale *i* in layer of skin *j* and in year *k*, α_j is a fixed intercept for layer *j*, a_i represents a random intercept for each individual whale *i*, a_k represents a random intercept for each year *k*, $\beta_{1,j}$ and $\beta_{2,j}$ are layer-specific coefficients that allow isotope ratios to change differently by layer over the course of the season (DOY) to capture potential lagged effects of diet changes across different layers, and the ε_{ij} are residuals. The layer-specific coefficients $\beta_{1,j}$ and $\beta_{2,j}$ were estimated by including an interaction term between layer and DOY. Seasonal trends in isotope ratios were modeled as a quadratic function of DOY based on preliminary analyses that strongly suggested a curvilinear seasonal pattern. The random effects a_i and a_k, and residuals ε_{ij} are assumed to be independent and normally distributed with mean zero and variances σ^2_{ai}, σ^2_{ak} and σ^2, respectively. The models were fit using maximum likelihood or restricted maximum likelihood (REML) as implemented in package "nlme" for R version 3.3.1 [92]. Models were initially fit using maximum likelihood estimation to allow for model selection using AICc values. Once the AICc-best model was identified, we re-estimated the final model using REML.

1.4 Results

1.4.1 Experiment 1

Stable carbon and nitrogen isotope ratios from the three cetacean species did not overlap in 2-D space: relative to the other species, the sperm whale had high $\delta^{15}N$ and $\delta^{13}C$ values, the fin whale had low $\delta^{15}N$ and $\delta^{13}C$ values, and the humpback whale had low $\delta^{15}N$ values and high $\delta^{13}C$ values (Table 1.3, Figure 1.2). Variability in $\delta^{15}N$ values, as measured by their standard deviation across all skin subsamples, was more than twice as high in the humpback whale than in the other species (Table 1.3). The sperm whale had the highest variability in $\delta^{13}C$ values (Table 1.3, Figure 1.2).

The fin whale samples did not differ significantly in $\delta^{15}N$ values or $\delta^{13}C$ values among cores or layers (Table 1.4, Figure 1.3). Layer was a significant predictor for one stable isotope ratio each for both the sperm whale ($\delta^{13}C$) and humpback whale ($\delta^{15}N$), while core was not significant for either species or isotope. The humpback whale samples had significantly different $\delta^{15}N$ values among layers ($F_{2,6}$=122, p<0.001; Table 1.4, Figure 1.3) and all pairwise differences were significant (p=0.043, p<0.001, p<0.001 for inner-middle, middle-outer, and inner-outer comparisons respectively). No significant differences were found in $\delta^{13}C$ values among layers for the humpback skin (Table 1.4). The sperm whale sample had significantly different $\delta^{13}C$ values among layers ($F_{2,6}$=11.9, p=0.008; Table 1.4, Figure 1.3). Specifically, the inner layer of sperm whale skin had significantly higher $\delta^{13}C$ values than both the middle (p=0.02) and the outer layers (p=0.01) (Table 1.4). No significant differences in $\delta^{15}N$ values among layers were found in the sperm whale skin (Table 1.4).

1.4.2 Experiment 2

Among the 28 individual sperm whales, stable isotope ratios showed broad ranges by layer. The range of $\delta^{15}N$ values within an individual averaged (±SD) of 0.40±0.22‰ (min 0.1; max 1.1), while the range of $\delta^{13}C$ values within an individual averaged 0.65±0.31‰ (min 0.1; max 1.0). Both $\delta^{13}C$ values and $\delta^{15}N$ values decreased between the inner and middle layer on average, but these differences were highly variable across the 28 individual sperm whales (Figure 1.4). While $\delta^{15}N$ values showed on average a 0.1‰ difference between the middle and outer layers,

differences between the middle and inner layers were 0.2‰ (Figure 1.4A). For $\delta^{13}C$ values, the outer layer decreased an average of 0.4‰ from the middle layer (Figure 1.4B).

The AICc-best model for $\delta^{15}N$ values included layer and day of the year (DOY) as predictors, without an interaction term (Table 1.5). A likelihood ratio test determined that the random effect of year did not significantly improve the fit of the model (*L.ratio*=5.68, *p*=1), hence it was not included in the final model, thereby effectively pooling data across years. The model suggests that $\delta^{15}N$ values in all three layers decreased sharply on average through the first part of the season to a minimum in mid-summer, before increasing again towards the end of the season (Figure 1.5A). Random whale-specific intercepts suggested that variability in mean $\delta^{15}N$ values among individual whales (σ_a=0.63‰) was much larger than residual variability within individuals (σ=0.21‰) as evident in a large spread around the mean seasonal trend. The best-fit model indicated layer and day of the year explained 92% of the variability in $\delta^{15}N$ values (R^2=0.92). Although the best model included differences in mean $\delta^{15}N$ values among layers, Tukey post-hoc tests showed no significant pairwise differences between layers (Middle-Inner *t*= -1.07, *p*=0.53; Outer-Inner, *t*= -0.64, *p*=0.80; Outer-Middle, *t*=0.43, *p*=0.90).

For $\delta^{13}C$ values, the best model chosen using AICc selection included layer and DOY as predictors (Table 1.5). As with $\delta^{15}N$ values, the random effect of year did not significantly improve the fit of the model (*L.ratio*=1.25e-12, *p*=1) and was excluded from the final model. Coefficients for all of the top-ranked models indicated that $\delta^{13}C$ values decreased significantly from the inner layer of skin to the outer layer (Figure 1.5B). The R^2 value for the best-fit model (R^2=0.86) suggested layer and day of the year could explain 86% of the variance in stable isotope ratios. Random variability in mean $\delta^{13}C$ values among individuals (σ_a=0.50‰) was much larger than residual variability within individuals (σ=0.25‰). Based on the reduced model, pairwise differences were significant between the inner and middle layer (*t*= -2.49, *p*=0.03) and between the inner and outer layers (*t*= -3.55, *p*=0.001), but not between middle and outer layers (*t*= -1.06, *p*=0.54).

1.5 Discussion

The results from experiment 1 provide preliminary support to the hypothesis of a dietary time series in layers of the skin of cetaceans [41], specifically in humpback and sperm whales. We found significant differences in stable isotope ratios among different layers but not among cores in both sperm and humpback whales, which are known for their isotopically diverse diets [61,66–71,73–75]. Furthermore, stable isotope ratios did not differ among layers or cores in the skin of the fin whale, consistent with our expectations for a species which is thought to have a less variable diet [19,61–65]. These results suggest that subsampling only the inner layer of cetacean tissue could provide a means for assessing recent diets, better suited for interpretation with observational feeding studies and comparative studies where prey is sampled as well.

While ANOVA results found that cores were not included in the best-fit model, there was variability in stable isotope ratios across layers (Figure 1.3). However, while systematic in the differences among layers, the variability within a layer (across cores) was minimal and random. Further, all but two of the ranges in $\delta^{13}C$ and $\delta^{15}N$ values within a layer were under 0.5‰, which was lower than the range of stable isotope ratios measured across 11 different skin positions in common and striped dolphins [39]. Other causes of variability within a layer, including the two outlier layers with high variability may be related to limitations in accuracy of cutting layers when sampling the skin. Increasing our sample size for each species would likely smooth out that variability. We have included subsampling within layers (cores) to serve as a control because we do not expect stable isotope values to vary *systematically* by core (we do have this expectation of values by layer). However, it is no surprise that there would be some random variation, which is what makes core a useful control, and thus the low variability in the stable isotope ratios of cores found in this study justifies the ANOVA results of non-significance, implying heterogeneity.

The stable isotope ratios in experiment 1 were within or near the range of what is to be expected from each of these species [1,2,19,53]. The lack of significant differences between cores or layers in the fin whale indicates that the whale did not undergo a marked diet-shift during the turnover time of the skin. This was expected given the fairly homogenous diet of this species [19,53,61]. The fin whale's $\delta^{13}C$ values were surprisingly low, 1- 2‰ lower than $\delta^{13}C$ values observed from other studies of fin whales [19,53,65]. However, two of these studies were conducted at either a much

lower latitude [53] or a combination of a lower latitude and different ocean basin [19], both of which can significantly affect $\delta^{13}C$ values [93–96]. In particular, higher latitude foraging is known to result in lower (more negative) $\delta^{13}C$ values in the North Pacific Ocean [93,94,96]. Witteveen & Wynne [65] found $\delta^{13}C$ values of near-shore fin whales in a similar region as this study to be on average 1.5‰ higher than this study. Given similar latitude in the same ocean basin, this suggests the fin whale in this study was foraging farther offshore. Stable isotope studies of zooplankton in Prince William Sound and the central GOA have shown strong patterns of lower $\delta^{13}C$ values offshore [97], though seasonal and annual differences and fluctuations do exist. This whale was struck by a cruise ship transiting the GOA, whereas samples from Witteveen & Wynne were collected near-shore close to Kodiak Island, perhaps resulting in higher $\delta^{13}C$ values [65].

The difference in stable nitrogen isotope ratios between the inner and outer layers of the humpback whale could indicate diet shifts from higher trophic level herring in the spring to lower trophic level krill in the summer before it died. Humpback whales in Southeast Alaska eat a mixed diet of herring, krill, and other forage fish [66–69,98], and have a lower trophic level diet overall than those in other areas of Alaska [2]. The humpback whale sampled in this analysis was found dead on July 28, 2010, and while deposition rates are unknown for this species, incorporation of stable isotopes from prey into tissue has been estimated in other cetacean species to occur between 2.5 to 6 months [41,42,59]. Herring spawn in Southeast Alaska in the spring, forming dense aggregations that attract large numbers of humpback whales as they return from their low-latitude breeding areas [70,98]. The outer layer of our sample, representing the least recent diet, shows the highest $\delta^{15}N$ value, which is consistent with that of a whale foraging primarily on herring or other schooling fish such as eulachon or pollock [29]. Krill are the most common humpback whale prey in Southeast Alaska and have their highest energy content in late summer and fall [67], which could explain the low $\delta^{15}N$ values in the inner layer of our sample that reflects prey consumption during the summer months.

The sperm whale's higher $\delta^{15}N$ values relative to the fin and humpback whales reflect its higher trophic position (Table 1.3, Figure 1.2). The sperm whale's higher $\delta^{13}C$ values can be attributed to foraging on higher trophic level species found farther offshore than the two baleen whales (Table 1.3, Figure 1.2). An increase in the $\delta^{13}C$ value of the inner layer of sperm whale skin of

just over 1‰ represents a trophic level difference and could indicate a recent shift to more benthic prey or higher-latitude prey [9,94,99,100]. Higher latitude feeding tends to result in higher $\delta^{13}C$ values in sperm whale skin in the southern hemisphere [52], which would be consistent with the inner layer of sperm whale skin in this study if the whale had been a recent arrival to Alaskan waters [94,99,101]. Conversely higher latitude has shown decreased $\delta^{13}C$ values in other studies, which would not be supported by this data [94]. Foraging on larger squid and foraging closer to shore results in higher $\delta^{13}C$ values in sperm whale tissue [94]. Finally, even if the whale had been foraging in the same Alaskan waters for an extended period of time, seasonal shifts in the isotopic baseline of the ecosystem could be responsible for the trophic level shift between layers [6,11,100]. The lack of significant differences in the $\delta^{15}N$ values of this individual could simply indicate that the trophic position of sperm whale prey between different foraging areas or during the timeline of the skin was not significantly different. There have been no comprehensive stable isotope analyses of prey species of sperm whales in the GOA to confirm this, but $\delta^{15}N$ values found in this study are similar to those measured from adult male sperm whales in the Gulf of California [1].

The specific dietary time period represented by each layer of skin remains unknown, and likely varies between species and regions. A recent study with blue whales has estimated full isotopic incorporation (turnover) in nitrogen isotopes ($\delta^{15}N$ values) of the largest cetacean and baleen whale to be 163±91 (mean±SD) days [41]. For odontocete species, the most comprehensive controlled feeding experiments have used bottlenose dolphins as a subject, one of which found 95% turnover achieved in 104±35 days for $\delta^{13}C$ values and 180±71 days for $\delta^{15}N$ values [42]. The results from these two studies is surprising given they estimated similar isotopic incorporation rates despite vast differences in size of the two cetaceans represented, the blue whale and the bottlenose dolphin [41,42]. For $\delta^{15}N$ values alone, assuming new skin is formed and sloughed at a constant rate, each layer (strata) of skin could be estimated to represent 54±52.5 or 68.7±49 days of dietary information in the blue whale and bottlenose dolphin studies respectively, and applied to other (e.g. sperm whale) species. It must be noted too that water temperature likely has an effect on epidermal turnover. Studies have shown both killer whales and beluga whales use seasonal migration to or through warmer waters to stimulate epidermal molt and skin sloughing [59,102]. The isotopic incorporation rates studied recently in blue whales between two different

foraging regions found very different isotopic incorporation rates between the regions (84±5 days for Gulf of California versus 242±44 days for the California Current) [41]. The authors hypothesized that cooler water temperatures in the California Current ecosystem could have slowed turnover rates in that region when compared to the warmer water of the Gulf of California.

For experiment 2, layers of skin from 28 individual sperm whales showed similar patterns to those found in the single sperm whale from experiment 1. However, experiment 2 also showed a surprising consistency in the patterns of isotopic differences among layers, highlighting the need for more research. The range of $\delta^{15}N$ values among layers in an individual averaged 0.4‰ (min=0.1‰; max=1.1‰), while the range of $\delta^{13}C$ values among layers averaged 0.7‰ (min=0.1‰; max=1.5‰). Given the variability of 0.1‰ found in replicate skin samples from a single humpback whale[35], these findings indicate that sperm whale skin reveals differences in isotopic values among layers that cannot be explained by within-tissue variability alone nor the precision of the mass spectrometer.

The presence of trends in stable isotope ratios among layers of different individuals found in mixed effects modeling of experiment 2 was not expected (Figure 1.4, 1.5). In spite of high variability among individuals, the inner layer of skin generally had higher $\delta^{15}N$ and $\delta^{13}C$ values than other layers throughout the summer season (Figure 1.5) with the pattern being more pronounced in the $\delta^{13}C$ values. This pattern persisted over time, across a significant seasonal trend where $\delta^{15}N$ values dropped mid-summer and increased again in late summer (Figure 1.5A) and $\delta^{13}C$ values showed a slight decrease earlier in the summer and then increased in late summer (Figure 1.5B). Had these animals all come to Alaska around the same time period in late spring, we would have expected the later-season samples to have outer and middle layers trending toward the inner layer (and inner layers of the early-season samples) as the full thickness of the skin began to represent their Alaskan foraging ground habitats. Similarly, if all animals had been experiencing the same seasonal shift in diet, we would again have expected later season samples to have outer and middle layers trending toward the inner layer. Finally, if animals had all been foraging in different areas prior to coming to Alaskan waters, or if they had been eating variable diets, we may expect layers to all exhibit differences individually, but we

would not expect those differences to carry the same trend, in that the inner layer of skin was consistently higher across multiple individuals taken over a 4 month time period. Instead the trend we see is that no matter when they were sampled (May through September), most sperm whales had higher $\delta^{13}C$ values in their inner layer than outer layer.

There are a few potential reasons we might see a trend in layers of sperm whale skin with consistently higher $\delta^{13}C$ values in the inner layer of skin across multiple individuals. It is possible that the turnover rate in sperm whales at high latitudes is extremely long, as Busquets-Vass *et al.* [41] hypothesized for blue whales, and each layer of skin represents a longer time period than the estimated 54-68 days mentioned above. Given Busquets-Vass *et al.*'s [41] estimation of 242±44 days for isotopic incorporation in the California Current region (the northernmost region of their study), each layer would represent 80±25 days, likely more for whales inhabiting even colder waters in the GOA. Indeed evidence suggests sperm whales slough large amounts of skin in Gulf of California waters [103], while anecdotal evidence from the authors' 15-year history of field research shows sloughed skin is rarely if ever seen in GOA waters. We propose sperm whale skin slows isotopic incorporation rates as they move to higher latitudes. With our range of sampling dates spanning from late May to mid-September (115 days total), our samples could conceivably represent one season (summer). Thus the inner layer of skin may represent a shift in diet, baseline isotope ratios, or large-scale movement for each whale individually to higher $\delta^{13}C$ values relative to their other layers. While each individual whale has its own "niche" of diet preference, the higher isotopic ratios in the inner layers may represent a large-scale, seasonal baseline shift from winter/spring to summer/fall signatures that affected all individuals regardless of sampling date.

Though the inner layer of skin may represent a longer time period spanning an entire summer, or nearly four months time, the specific causes of higher $\delta^{13}C$ values in the inner layer of skin remain inconclusive. Studies on plankton and squid have shown $\delta^{13}C$ values generally decrease with increasing latitude [93,101], though they may slightly increase again at latitudes north of 40°N [94]. This pattern is opposite what we would expect if the inner layer of our samples represented the result of a migration from warmer equatorial waters to Alaskan waters in the summer. It must be noted that sperm whales have a higher trophic level than species in these studies, and would not be an ideal tissue to infer baseline shifts because trophic discrimination likely obscures

changes in baseline patterns. Additionally, while male sperm whales are generally thought to move to GOA waters in the spring and summer months, no pattern exists for sperm whale departures from Alaskan waters [104], suggesting arrivals are similarly non-patterned. Shifting prey species between regions could also explain changes in stable isotope ratios, as sperm whales in the eastern GOA are known to consume more deep sea fish than squid, unlike other regions off the western coast of North America where squid is the primary diet item [1,61,73,74,105]. While stable isotope ratios of prey in the eastern GOA are largely unknown, $\delta^{13}C$ values of squid in the California Current region increase with size [1] and are generally higher than $\delta^{13}C$ values of deep sea fish in that region [11]. This pattern is again opposite what would be expected if the inner layer of skin represented diet from the GOA versus diet from lower latitude habitats. Together, these potential isotopic shifts that would result in opposite patterns in the layers of sperm whale skin than was observed in this study signal that there is much still to be learned about how stable isotopes move through ecological systems and are incorporated into consumer tissues.

The observed consistent stable isotope gradient among individuals could also be due to improperly extracted lipids or structural differences among layers, but we consider these explanations to be unlikely. Differences in lipids among different layers of the skin may not be properly accounted for in the analyses. C:N ratios can be an indicator of carbon content, and are used as a metric for lipid extraction in studies where high lipid content is inherent to the tissue. [82,106] We are confident that our lipid-extraction methods were not the cause of the trends observed in $\delta^{13}C$ values between layers. We used standard lipid-extraction techniques [84,88,89], and found $\delta^{13}C$ values to vary significantly between non-lipid-extracted and lipid-extracted values. There was also no trend in $\delta^{13}C$ values when plotted against the C:N ratios of our lipid-extracted samples. The literature varies when discussing C:N ratios of sufficiently lipid-extracted tissues with appropriate values between 3.5 and 4 and our values ranged from 2.9 to 3.8 falling within the range of other studies [21,81,82,87]. In addition, if lipid-extraction was not complete, we would expect the inner layer, nearest to the blubber, to have a lower $\delta^{13}C$ value than other layers, rather than higher. Furthermore, differences between layers were not completely consistent across individuals (Figure 1.4B), including some individuals with lower $\delta^{13}C$ values in the inner layer. These differences among individuals suggest that the patterns we are seeing are not due to structural differences in the layers of skin that might affect isotope ratios.

Within the consistent pattern of higher delta values in the inner layer of sperm whale skin, there was also a significant seasonal gradient for both $\delta^{13}C$ and $\delta^{15}N$ values. The interpretation of this seasonal gradient across the sampling period of May to September also depends on isotopic incorporation rates, and the temporal snapshot of each layer. A longer incorporation rate as suggested above, with the inner layer representing a 4-month period, would potentially indicate a lagged dietary shift, where the inner layer of skin is responding to foraging changes some time before they appear in the skin. Rather than a single factor driving the patterns of change over time that were observed, it is more likely a combination of seasonal and regional diet shifts, changes in isotopic baselines, and more nuanced movement behaviors that influence the stable isotope ratios observed in this study.

The differences in seasonal trends among layers indicates some trends may also be masked by the high variability among individual whales and by the uneven distribution of samples across the season. The nature of the opportunistic sampling for sperm whale tissues resulted in the earliest sampling date of late May, and the latest sampling date of mid-September, representing 3.5 months or around ¼ of a year. Within this relatively short sampling season, the majority of samples were collected in July (Figure 1.5). With a turnover rate in high latitudes that is potentially upwards of 242 days ($^2/_3$ of a year), sampling whales early in the season (March & April) as well as later in the season (October & November), and re-sampling the same individuals over the course of a season would shed more light on the specific seasonal drivers of diet for these animals. Additionally, increased knowledge of prey characteristics and availability in these habitats would be useful to better understand sperm whale foraging habits in this region.

1.6 Conclusion

In light of these experiments and findings of variable stable isotope ratios among layers of cetacean skin, we suggest this pilot study provides conditional evidence that cetacean skin shows a dietary time series. Directed sampling of specific layers of skin across a population may allow for more nuanced analysis in dietary trends of whales. However, we also contend that attributing trends in observed stable isotope ratios to specific ecological systems is more nuanced than may have been previously thought. Above all, this study supports the argument that in stable isotope

analysis of cetacean skin it is important to select a portion of skin to analyze based on the species and objectives of the study. Due to structure of skin, there may be a significant temporal gradient between inner and outer layers of skin. For studies focused on diet in species where foraging preference may change over time or the species may migrate, the inner layer of skin may give the most precise information to compare with prey and ecosystem baselines or in mixing models to assess prey contribution. However, for studies focusing on general isotopic niche and trophic level calculations, a full-thickness homogenized sample of skin may provide a more appropriate long-term average of diet composition for the species. Sloughed skin samples may be useful as an opportunistic sampling method for species that have a more general diet, do not exhibit prey switching, and/or do not migrate. Most importantly, further research with cetacean skin should seek to expand on this research to examine specifically how stable isotopes move through the tissue, and at what temporal scales. Overall, it is imperative that researchers communicate the portion of skin used in the study to allow the scientific community to better assess conclusions taken from isotope studies.

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1.9 Figures

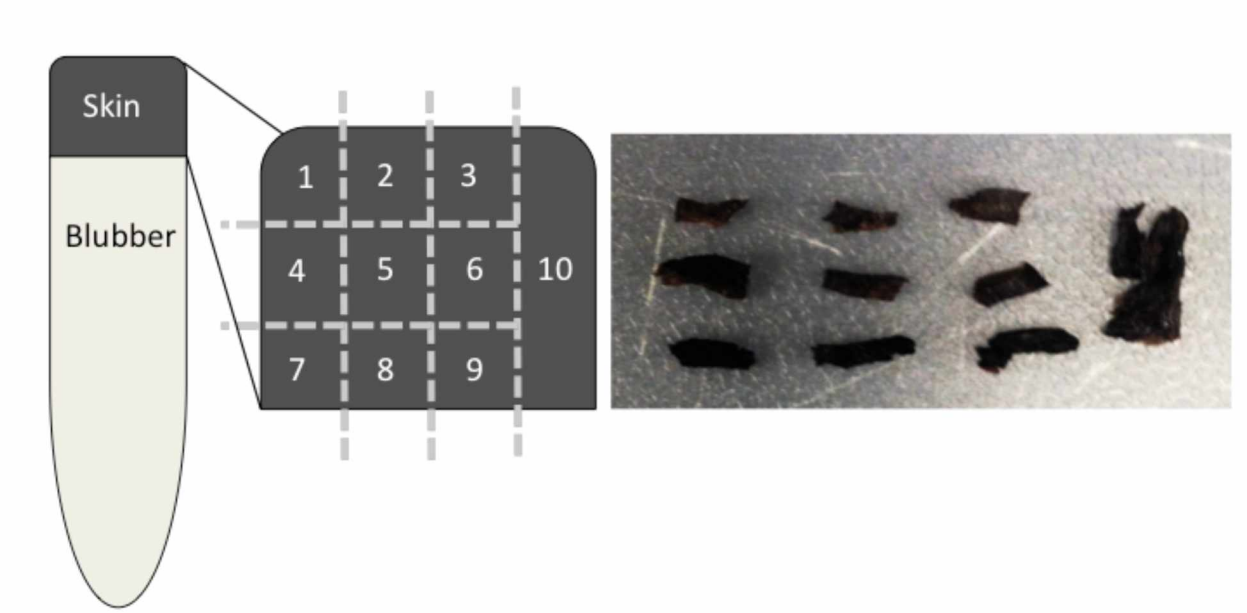

Figure 1.1 Left, schematic showing the methodology for sub-sampling a biopsy sample into 10 subsamples of skin to analyze differences within layers (1-3, 4-6, 7-9) and within cores (1,4,7 vs. 2,5,8 vs. 3,6,9). Right, photo showing sperm whale skin divided into 10 sections, as defined on the left.

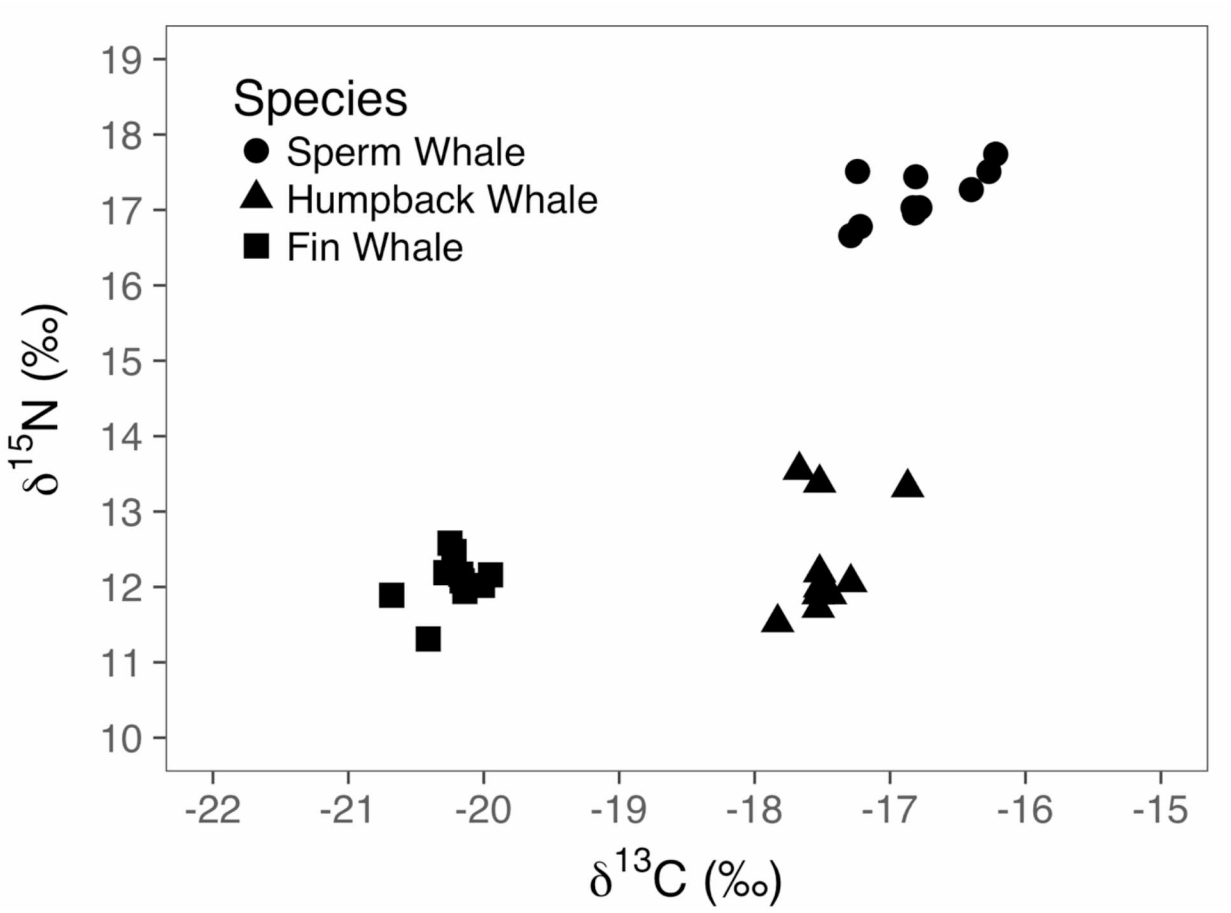

Figure 1.2 Bi-plot showing delta values of stable carbon and nitrogen isotope ratios of the 10 subsamples of each of the three species of whale sampled in this experiment.

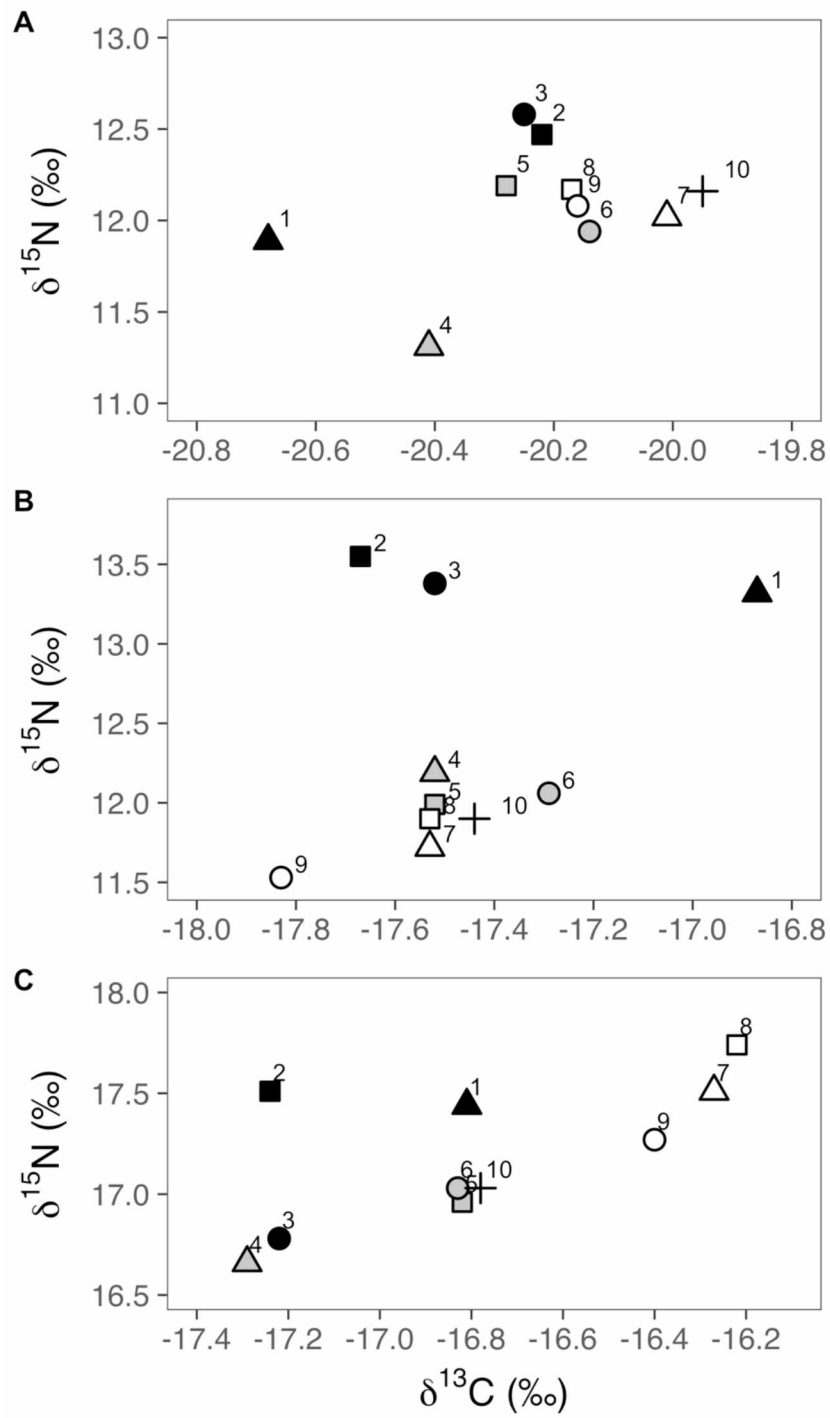

Figure 1.3 Bi-plot showing delta values from each piece of tissue from the fin (A), humpback (B), and sperm (C) whale samples that were sub-sampled into 10 pieces (Figure 1.1). Layers are shaded the same with outer layer = black, middle layer = grey, and inner layer = white. Cores are indicated with shapes, from left to right = triangles, squares, and circles. Sample 10, the full sample, is shown in with a "+" symbol. Note differences in scale of x-axes.

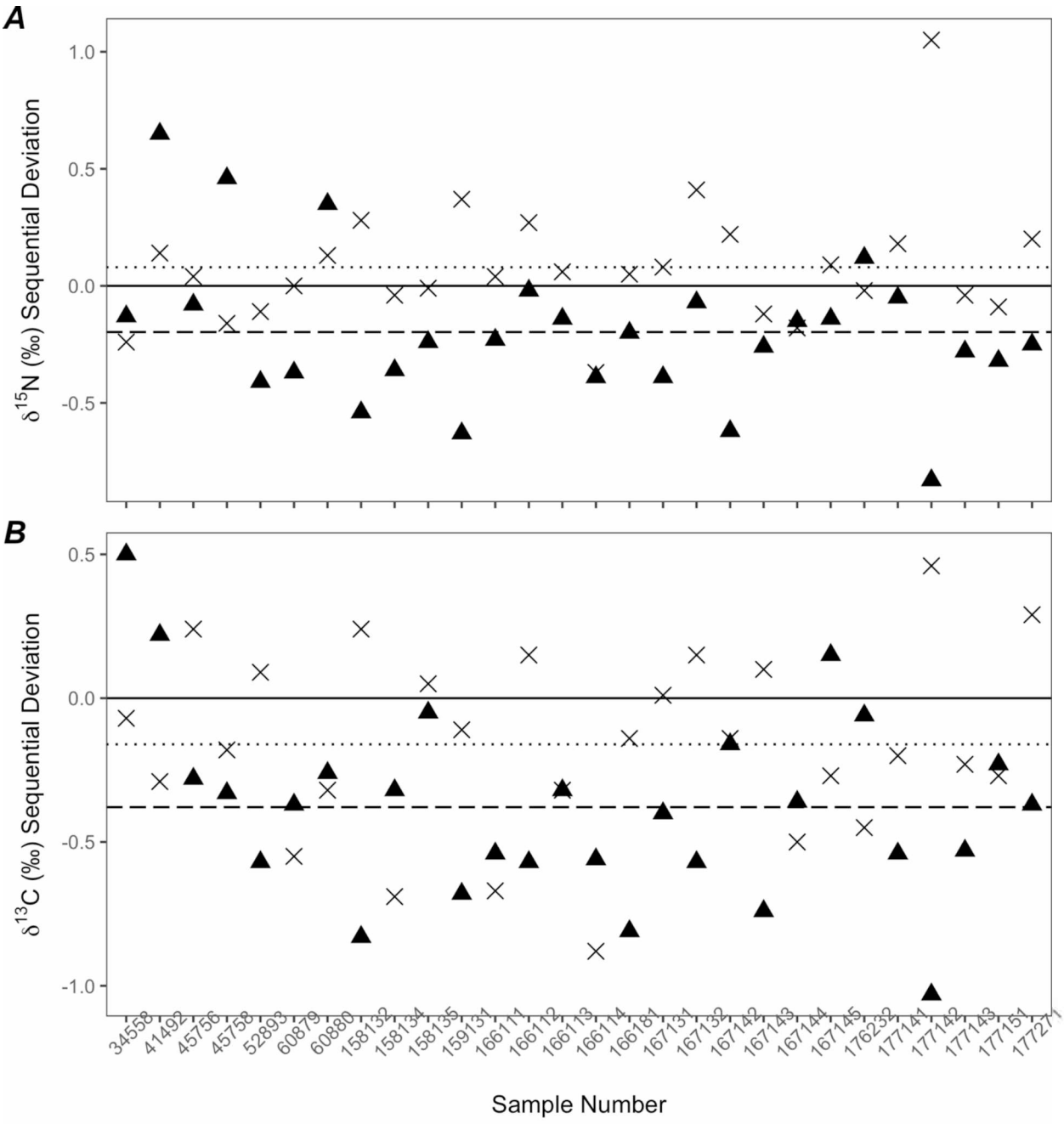

Figure 1.4 Twenty-eight sperm whale skin samples showing sequential differences in $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values between layers. Differences between the middle and inner layer (▲) are on average (dashed line) greater than differences between the middle and outer layers (×, dotted line).

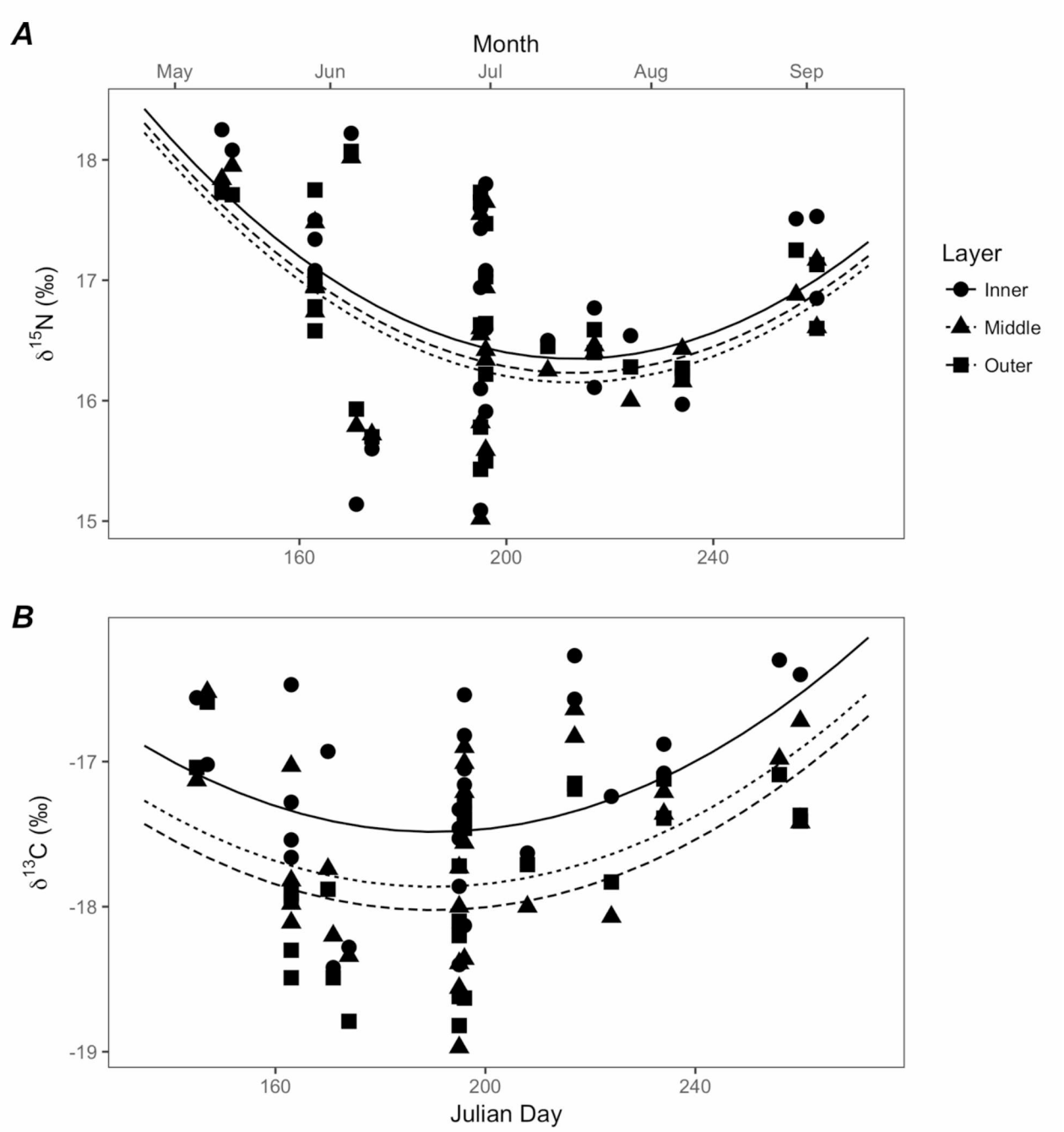

Figure 1.5 Observed sperm whale $\delta^{15}N$ (A) and $\delta^{13}C$ (B) values for each layer with respect to Julian day of the year, and predicted means for each layer of skin (solid line = inner, dotted line = middle, dashed line = outer) based on best-fit models.

1.10 Tables

Table 1.1 Existing experiments addressing turnover rates of cetacean skin

Species	Method	Estimated turnover rate (days, $\bar{x} \pm$ sd)	Type of turnover	Source
Blue whale (*Balaenoptera musculus*)	Free-ranging with known migratory diet shift	163 ± 91 ($\delta^{15}N$)	Full isotopic incorporation	Busquets-Vass *et al.* 2017 (Busquets-Vass *et al.*, 2017)
Bottlenose dolphin (*Tursiops truncatus*)	Labeled cells sub-epidermally	73	Full epidermal turnover	Hicks *et al.*1985 (Hicks *et al.*, 1985)
Bottlenose dolphin (*Tursiops truncatus*)	Controlled feeding	17 ± 1.3 ($\delta^{15}N$) 22 ± 0.5 ($\delta^{13}C$)	Half life	Browning *et al.* 2014 (Browning *et al.*, 2014)
Bottlenose dolphin (*Tursiops truncatus*)	Controlled feeding	180 ± 71 ($\delta^{15}N$) 104 ± 35 ($\delta^{13}C$)	Full isotopic incorporation	Giménez *et al.* 2016 (Giménez *et al.*, 2016)
Beluga (*Delphinapterus leucas)*	Labeled cells sub-epidermally	70-75	Full epidermal turnover	St. Aubin *et al.* 1990 (St. Aubin *et al.*, 1990)

Table 1.2 Types of samples used for experiments 1 and 2. F = fin whale, H = humpback whale, and S = sperm whale. Sample size is in parentheses.

	Stranded (Sample Size)	Live free ranging (Sample Size)
Experiment 1	F (1) H (1)	S (1)
Experiment 2	-	S (28)

Table 1.3 Means and standard deviations (sd) of $\delta^{13}C$ and $\delta^{15}N$ values and C:N ratios for each layer and overall of the three cetacean species sampled in experiment 1.

	Mean $\delta^{13}C$	s.d. $\delta^{13}C$	Mean $\delta^{15}N$	s.d. $\delta^{15}N$	Mean C:N	s.d. C:N
Sperm whale						
Outer	-17.2‰	0.2‰	17.2‰	0.4‰	3.6	0.1
Middle	-17.0‰	0.3‰	16.9‰	0.2‰	3.4	0.2
Inner	-16.2‰	0.1‰	17.5‰	0.2‰	3.1	0.1
Overall	-16.8‰	0.5‰	17.2‰	0.4‰	3.4	0.2
Humpback whale						
Outer	-17.4‰	0.4‰	13.4‰	0.1‰	3.3	0.1
Middle	-17.4‰	0.1‰	12.1‰	0.1‰	3.3	<0.1
Inner	-17.6‰	0.2‰	11.7‰	0.2‰	3.2	<0.1
Overall	-17.5‰	0.3‰	12.4‰	0.8‰	3.3	0.1
Fin whale						
Outer	-20.4‰	0.3‰	12.3‰	0.4‰	3.6	0.1
Middle	-20.3‰	0.1‰	11.8‰	0.5‰	3.6	0.1
Inner	-20.1‰	0.1‰	12.1‰	0.1‰	3.5	0.1
Overall	-20.2‰	0.2‰	12.1‰	0.4‰	3.5	0.1

Table 1.4 Results from ANOVA tests for experiment 1 of stable isotope analysis of sperm whale, humpback whale, and fin whale cores and layers. Bold model results indicate significant relationships for each species and isotope ratio.

Species	Factor	F-value	*p*-value
Sperm Whale			
$\delta^{13}C$	**Layer**	**$F_{2,6}$=11.91**	***p*<0.01**
	Core	$F_{2,6}$=0.01	*p*=0.99
	Layer + Core	$F_{4,4}$=4.06	*p*=0.10
$\delta^{15}N$	Layer	$F_{2,6}$=3.44	*p*=0.10
	Core	$F_{2,6}$=0.72	*p*=0.52
	Layer + Core	$F_{4,4}$=2.68	*p*=0.18
Humpback Whale			
$\delta^{13}C$	Layer	$F_{2.6}$=0.79	*p*=0.50
	Core	$F_{2,6}$=0.87	*p*=0.47
	Layer + Core	$F_{4,4}$=0.76	*p*=0.60
$\delta^{15}N$	**Layer**	**$F_{2,6}$=122.7;**	***p*<0.01**
	Core	F2,6=0.02	*p*=0.98
	Layer + Core	$F_{4,4}$=60.14	*p*=0.05
Fin Whale			
$\delta^{13}C$	Layer	$F_{2,6}$=1.79	*p*=0.24
	Core	$F_{2,6}$=0.69	*p*=0.54
	Layer + Core	$F_{4,4}$=1.29	*p*=0.41
$\delta^{15}N$	Layer	$F_{2,6}$=1.62	*p*=0.27
	Core	$F_{2,6}$=2.67	*p*=0.15
	Layer + Core	$F_{4,4}$=4.59	*p*=0.09

Table 1.5 Results from linear mixed effects model selection for Experiment 2, identifying variables that influence $\delta^{15}N$ and $\delta^{13}C$ values in layers of skin of 28 sperm whales. Table shows the top five models for each isotope ratio. "DOY" refers to the day of the year variable, which is also included in the model as a quadratic term, and is allowed to interact with the layer variable. The symbol "+" indicates the factor variable is included in the model. Weights refer to AICc weights from each model.

	Model Rank	DOY^2	Layer	DOY* Layer	df	logLik	ΔAICc	Weight
$\delta^{15}N$	1	+	+		8	-36.05	0	0.81
	2		+		6	-40.36	3.81	0.12
	3	+			6	-41.47	6.02	0.04
	4	+	+	+	12	-34.35	7.09	0.02
	5				4	-45.79	10.10	0.01
$\delta^{13}C$	1	+	+		8	-37.21	0	0.75
	2		+		6	-40.76	2.28	0.24
	3	+	+	+	12	-36.52	9.10	0.01
	4	+			6	-60.28	41.32	0
	5				4	-63.84	43.85	0

Chapter 2 Exploring variability in the diet of depredating sperm whales in the Gulf of Alaska through stable isotope analysis[2]

2.1 Abstract

Sperm whales interact with commercially important groundfish fisheries offshore in the Gulf of Alaska (GOA). This study aims to use stable isotope analysis to better understand the trophic variability of sperm whales and their potential prey, and to use dietary mixing models to estimate the importance of prey species to sperm whale diets. We analyzed tissue samples from sperm whales and seven potential prey (five groundfish and two squid species). Samples were analyzed for stable carbon and nitrogen isotope ratios, and diet composition was estimated using Bayesian isotopic mixing models. Mixing model results suggest that an isotopically combined sablefish/dogfish group, skates, and rockfish make up the largest proportion of sperm whale diets (35%, 28% and 12%) in the GOA. The top prey items of whales that interact more frequently with fishing vessels consisted of skates (49%) and the sablefish/dogfish group (24%). This is the first known study to provide an isotopic baseline of adult male sperm whales and these adult groundfish and offshore squid species, and to assign contributions of prey to whale diets in the GOA. This study provides information to commercial fishermen and fisheries managers to better understand trophic connections of important commercial species.

2.2 Introduction

Understanding top predator diets and their role in marine food webs is important to managing fisheries and mammals from an ecosystem perspective. Sperm whales (*Physeter macrocephalus*) are the largest of the toothed whales, a deep diving cosmopolitan species inhabiting the world's major oceans. Females and calves primarily inhabit warm equatorial regions between 40°S and 40°N latitude, while males are thought to leave their natal groups after age 12, when they move to high latitude feeding grounds and roam widely [1,2]. One of those high latitude foraging grounds is in the Gulf of Alaska (GOA), where sperm whales were historically killed in large numbers during commercial whaling [3,4]. An estimated 157, 680 sperm whales were killed in

[2] Wild, L.A., Mueter, F.J., Witteveen, B.H., and Straley, J.M. 2020. Exploring variability in the diet of depredating sperm whales in the Gulf of Alaska through stable isotope analysis. Royal Soc. Open Sci. 7: 191110.

the North Pacific Ocean between 1948-1972 by Russian whaling ships alone [4], at a time when there was thought to be a population of 1,260,000 whales in the whole area [2]. A current reliable population estimate for the entire North Pacific Ocean does not exist, nor does one exist for the GOA itself. Within the GOA however, a line-transect survey in the western GOA estimated an abundance of 345 (CV=0.43) sperm whales in 2015 [5], and a mark-recapture abundance estimate for the eastern GOA in 2014 estimated 135 (95% CI: 124,153) individuals [6].

2.2.1 Sperm whale historical diet

Sperm whales primarily consume cephalopods worldwide, but in some parts of the world, including British Columbia, New Zealand, and Antarctic waters, fish are a considerable portion of the diet for males [1,7–11]. Stomach content data from scientists on whaling ships in Alaskan waters in the 1960s indicate sperm whale diets were dominated by squid in the Bering Sea and western GOA, but as whaling ships moved into the eastern GOA and off the northern British Columbia coast, stomachs contained a majority of fish remains [8,12–14]. Of these fishes, the most common occurrences in sperm whale stomachs noted in the "Northeast Pacific" and from northern British Columbia whaling stations between 1936 and 1967 were ragfish (*Icosteus aenigmaticus*), rockfish (*Sebastes spp.*), skates (*Rajidae spp.*), and dogfish (*Squalus suckleyi*) [8,11,14,15]. Of the squid remains collected from sperm whale stomachs in the North Pacific, including the GOA and British Columbia coast, the primary species found were robust clubhook squid (*Onykia robusta*) and magister armhook squid (*Berryteuthis magister*) [11,12,14–16]. Interestingly, sablefish as a diet item were only mentioned once in stomach contents data from northern California whaling stations [8].

Since the rapid decline in commercial whaling following the International Whaling Commission's moratorium in 1986, studying the diet of large free-ranging cetaceans has become more difficult, as stomach samples from these animals are no longer readily available and are limited to stranded animals, which is rare in a species that generally lives far from shore. In fact, there appear to have been no publications of collections or analysis of stomach contents from stranded sperm whales in the GOA or Northeast Pacific Ocean in the last forty years. Collection of feces can give insight into diet but requires opportunistic detection and collection; a whale has to be observed defecating, and feces must be collected before it dissipates and sinks. Thus few

studies exist for sperm whales that include analysis of feces, and none that we found for the GOA or Northeast Pacific Ocean in recent history. Direct observations of feeding sperm whales are not practical as they forage and consume prey at depth. Overall, while these methods can provide a snapshot of recent diet, they suffer from low sample sizes, high cost of collection, potential bias related to cause of death for stranded animals, and bias towards hard parts from species that are less likely to break down in the stomach and can therefore be identified in stomachs or feces.

2.2.2 Sperm whales in the Gulf of Alaska

Today, sperm whales are still found in the GOA, where they are known to remove sablefish (*Anoplopoma fimbria*) from commercial longline fishing vessels [6,17,18]. This removal of caught fish, known as depredation, has been observed worldwide on multiple fish species [19,20], and in the GOA has increased since the implementation of the catch share or individual fishing quota program in the mid-1990's [6,18]. The economic impacts of depredation to the longline fishing fleet in the GOA are nuanced, and result in added costs for fishermen in fuel, bait, crew, and gear to make up for fish lost to whales [6,21,22].

Since 2003 the Southeast Alaska Sperm Whale Avoidance Project (SEASWAP) has been studying sperm whale depredation of longline fishing gear as a collaborative effort between scientists, fishermen, and fisheries managers, with the goal to minimize interactions between whales and fishing gear. SEASWAP effort is primarily based out of Sitka, on the outer coast of Southeast Alaska, and most research focuses on the eastern GOA study area, though depredation by sperm whales extends into the central and western GOA as well. Initial work found that whales cue in to the unique patterns of propeller cavitation made by longline vessels as they shift in and out of gear to stay on top of the line as it is hauled to the surface [23]. Whales can pick up these vessel-hauling sounds acoustically from at least 8-13 km away [24], with anecdotal evidence from research cruises showing detection of vessels on a calm day upwards of 16 km (J. Straley, unpublished data). Through acoustic and visual observations, SEASWAP has found that whales surfacing within 500 m of a fishing vessel hauling gear can be considered engaging in depredation activity [25], a metric that has been independently estimated in other parts of the world [19,20,26]. SEASWAP has only observed male sperm whales interacting with longline

fishing gear, which has been corroborated by genetic analysis of tissue samples collected from individual whales [27]. This finding is consistent with historical records in which Russian scientists on whaling ships noted male-only bachelor groups in the eastern GOA, along the continental slope [3].

2.2.3 Stable isotopes

Stable isotope analysis has become a useful tool to estimate recent diet composition, trophic position and food web connections, as well as to construct time series of dietary estimates [28–30]. The primary isotopes used in food web studies are carbon and nitrogen. Stable carbon isotope ratios (ratio of ^{13}C:^{12}C isotopes in a tissue, with respect to an international standard) reflect photosynthetic pathways of an animal's food sources [29]. Therefore, in the marine environment, stable carbon isotope ratios ($\delta^{13}C$), can show differences between benthic and pelagic foragers, between near-shore and offshore foragers, and between freshwater and saltwater foragers [30–32]. Additionally, they can reflect latitudinal gradients across ocean basins [33,34]. Stable nitrogen isotope ratios (ratio of ^{15}N:^{14}N isotopes in a tissue, with respect to an international standard) are considered a proxy for trophic level, as nitrogen isotopes experience metabolic fractionation as they move up the food chain from prey to predator [29,35,36]. Consequently, a predator's stable nitrogen isotope ratio ($\delta^{15}N$) will be higher than that of its prey in a process known as trophic enrichment.

Tissue-specific stable isotope ratios from predators (mixtures) and their prey (sources) can be used in isotopic mixing models to evaluate proportional contribution of prey to predator diets [37–41]. For cetacean species, a variety of tissues such as teeth, baleen, skin, muscle, and blubber have been sampled and analyzed using stable isotope analysis. While baleen, teeth, and muscle must be collected postmortem and therefore pose the same problems as collection of stomach contents, skin and blubber can be collected from free-ranging animals. Biopsy sampling of cetaceans is a minimally invasive technique that does not require handling or capturing of animals, and results in collection of a small amount of skin and blubber that can be used for a variety of genetic, metabolomic, and isotopic analyses [42,43].

2.2.4 Objectives of present study

The current diet of sperm whales in the GOA is poorly understood. The goal of this project was to better understand trophic position and foraging ecology of sperm whales and assess the importance of sablefish in modern sperm whale diets. To do this, our main objectives were to: 1) use stable isotope analysis to describe isotopic variability and calculate trophic position of male sperm whales and their prey in the GOA; and 2) use isotopic mixing models to assess the proportion of various prey to sperm whale diets in the GOA, and compare to historical stomach contents data where possible.

2.3 Methods

2.3.1 Data collection – whale biopsies

A total of 33 biopsy tissue samples were used in this experiment, collected from sperm whales during SEASWAP research activities from 2003 to 2017 (Figure 2.1). Three samples, collected from the NOAA GOA longline survey in 2006, were taken in the central GOA, outside the study area (Figure 2.1). However, these samples fell within $\delta^{13}C$ and $\delta^{15}N$ values of other samples collected in the eastern GOA, and isotope ratios were not significantly different than the rest of the samples (MANOVA, $F_{8,56}$=2.19, p=0.05), and so they were included in the analysis to help improve our sample size. Samples were collected under NOAA research permits #14122 and #18529, and Institutional Animal Care and Use Committee project #906340-4. These permits ensure the safe and humane interactions between researchers and animals. Sperm whales were not captured or handled at all during collection of biopsy tissues, and no animals were harmed or killed during the study. Biopsies were collected at a distance of 15m or greater, using a modified Barnett crossbow equipped with a 40mm stainless steel tip [42]. Animal reactions were recorded before, during, and after the samples were collected; all animals resumed pre-sample behavior within one dive cycle after the samples were collected. Inner layer of skin (approximately 2mm thickness) was used in this analysis, likely representing the most recent diet of whales [44].

2.3.2 Data collection – prey

Fish and squid species historically found in sperm whale stomachs, as well as other species bycaught by commercial longline gear and available to depredating sperm whales, were analyzed

for stable isotopes (Figure 2.1, Table 2.1). Samples were collected and donated from a variety of sources, including commercial longline fishermen fleet members, the NOAA GOA longline survey, and the NOAA GOA bottom trawl survey (Table 2.1). Fish identified in historical stomach contents studies were requested from these sources and included ragfish, rockfish, spiny dogfish, and skates. Specific species of rockfish were not identified in whaling literature; therefore, we chose shortraker rockfish (*Sebastes borealis*) due to their large biomass relative to other rockfish species in the region and their prevalence in sablefish fishing habitat, suggesting they may be more available to whales from longline gear bycatch. Similarly, specific species of skates were not identified in whaling literature, so we requested all skate species bycaught on sablefish longline gear. All skate samples came from the NOAA GOA longline survey (Table 2.1), and only longnose skate (*Raja rhina*) were identified to species by NMFS scientists collecting the samples; all other species were grouped as "other" skates (specific species were not listed) though NMFS scientists collecting the samples noted that they consisted primarily of Alaskan and Big skates. Robust clubhook squid (*Onykia robusta*) and magister armhook squid (*Berryteuthis magister*) were reported in historical stomach contents and samples were requested and collected from commercial longline fishermen and the NOAA Bottom Trawl survey (Table 2.1). Species that were not identified in historic stomach contents but were included due to their prevalence as bycatch on longline fishing gear and subsequent availability to sperm whales while depredating were giant grenadier (*Albatrossia pectoralis*) and Pacific grenadier (*Coryphaenoides acrolepis*). While difficult to observe direct feeding, sperm whales have been observed following closely behind longline vessels and abruptly dipping underwater after grenadier have fallen from a hook near the surface of a longline haul (J. Straley, unpublished data).

We attempted to collect ragfish for this experiment due to their prevalence in the diets of sperm whales killed off the northeast coast of Vancouver Island and the eastern Gulf of Alaska during commercial whaling [8,11,12,14,15]. However, we were able to obtain only three specimens for this study, all of which were caught in shallow (< 50 m) waters well outside of our study area (Figure 2.1). Given the small sample size, none of which were collected in the study area, and coupled with a lack of any specimens found in habitat sperm whales are observed in or known to inhabit, we chose not to include them in the final analysis. However, we did run the full analysis

with the three ragfish included, and it did not change our results (Appendix A2.1 and Appendix A2.2).

2.3.3 Data collection – baseline organisms

Isotopic baselines, typically defined as the isotopic signature of primary consumers, vary between ecosystems, can fluctuate over years, and are crucial to accurate trophic level calculations [32]. In the offshore pelagic environment calanoid copepods (*Neocalanus sp.*) have been used [32,45]. For this study we collected and sorted *Neocalanus spp.* copepods from 200m depth bongo tows (mesh size 505µm) conducted offshore in the eastern GOA in July of 2016 and 2017 during NMFS GOA research surveys [46]. In 2016 and 2017, four and six stations were sampled, respectively, over the continental slope in water depths of 400m – 800m. For each sample, copepods were sorted by species, with *Neocalanus plumchrus* and *Neocalanus flemegeri* having the highest abundances. Due to their similar size and life history, they were combined, and approximately 10 specimens were selected from each station for isotope analysis.

2.3.4 Stable isotope analysis

For each squid specimen a 2-3 cm section of mantle and tentacle were sampled. For rockfish, dogfish, and grenadier, a 2-3 cm section of dorsal muscle was sampled. Muscle samples from the neck or "collar" area of sablefish were collected in order to retain the value of the fillet. A 2-3 cm section of skate muscle was sampled from the wing. Depth stratum (201-300m, 301-400m, 401-600m, 601-800m, and 801-1000m), length, and location (latitude, longitude) were recorded for each specimen, with the exception of clubhook squid, which were primarily collected from commercial fishermen who did not always record an exact location or depth. All samples were stored at -80°C until processing. All whale, squid, and fish samples were first rinsed with de-ionized water, then cut into small pieces and oven-dried at 60°C for 24 hours before being ground to a fine powder. Copepods were left whole. Lipids are known to be depleted in ^{13}C relative to ^{12}C, causing tissues with high lipid content to show falsely decreased $\delta^{13}C$ values [35]. However, the same is not true for $\delta^{15}N$. Therefore, to account for differences in lipid content, samples were lipid-extracted prior to analysis. However, half of the sample was first saved to run separately for $\delta^{15}N$ values to alleviate any changes to $\delta^{15}N$ values caused by lipid extraction [47–51]. Lipid extraction was carried out using three cycles in a 2:1 chloroform-methanol solution

[44,52–54]. Samples were then again dried overnight, after which 0.2-0.4mg aliquots were measured into tin capsules and sent to the Alaska Stable Isotope Facility (ASIF) in Fairbanks, Alaska. Samples were processed at ASIF with an elemental analyzer isotopic ratio mass spectrometer (EA-IRMS) for bulk carbon and nitrogen, expressed in delta (δ) notation, according to the equation:

Equation 1) $$\delta X(‰) = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000$$

where the isotope ratio X represents ^{13}C or ^{15}N, and R represents the abundance ratio of each isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Duplicates were run on 15 sperm whales and on 10 of each prey item except clubhook squid, for which 5 duplicates were run. Stable isotope ratios are expressed in units of parts per thousand ('per mil', ‰). Reference standards used were Vienna Pee-Dee Belemnite for carbon and atmospheric N_2 for nitrogen. Analytical precision was ±0.2‰ and ±0.3‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively, calculated from replicates of peptone and duplicate samples.

2.3.5 Trophic enrichment factors

Stable isotope ratios of materials change as they are incorporated into a consumer's tissues; the difference in isotope values between predator and prey is known as a Trophic Enrichment Factor (TEF), and can vary between species and between tissues within a single species [55]. Trophic level calculations, as well as dietary mixing models, require estimates of these rates. For marine mammals, estimation of TEFs can be complicated because direct control of feeding and/or continual observation of free-ranging marine mammals is nearly impossible. Thus many large whale species have estimated TEFs [56–58], while for pinnipeds and smaller cetaceans, captive feeding experiments have been performed [59–61]. TEFs in the skin of free ranging fin whales and pilot whales have been estimated for $\delta^{15}N$ values at 2.8‰ and 1.7‰ respectively and $\delta^{13}C$ values at 1.3‰ and 1.2‰ respectively [56,57]. Two captive feeding experiments exist where groups of bottlenose dolphins were tested under different diets [59,60]. We combined raw data from these captive feeding experiments and calculated variance-weighted mean TEFs and their standard errors as inputs for mixing models (TEF = 2.12 ± 0.53‰ for $\delta^{15}N$; TEF = 0.96 ± 0.38‰

for $\delta^{13}C$). Given our estimates were roughly mid-way between the fin whale and pilot whale free-ranging estimates, we ran separate analyses using both the low (pilot whale) and high (fin whale) TEF estimates as a sensitivity analysis (Appendix A2.4). Dietary proportions were slightly different, but relative importance of different prey items remain unchanged.

2.3.6 Objective 1: Describe isotopic variability and calculate trophic position

Isotopic variability

The mean (± SD) and range of isotopic variability was calculated for sperm whales and all prey species. For prey that had been split into two species, such as the longnose sakes and "other" skates, as well as the Pacific grenadier and giant grenadier, isotopic ratios between the groups were explored using analysis of co-variance (ANCOVA). This allowed us to explore how similar species were, and to determine whether or not the species should be grouped together for final dietary mixing model analysis or remain separated. Isotope ratios for each prey species were tested using ANCOVAs with respect to length and depth strata to assess whether prey should be split up in other ways from an isotopic perspective.

Trophic level calculation

The trophic level calculation is represented by:

Equation 2) $$TL = 2 + \frac{(\delta^{15}N_{specimen} - \delta^{15}N_{primary\ consumer})}{2.12}$$

where $\delta^{15}N_{specimen}$ represents the nitrogen isotope ratio values of the predator (i.e. sperm whales, fish, squid, etc.), $\delta^{15}N_{primary\ consumer}$ represents those of the baseline (calanoid copepods), 2 represents the assumed trophic position of the baseline consumer (*Neocalanus sp.*), and 2.12 represents the average enrichment per trophic level for whales. For trophic level calculations of fish and squid, a TEF of 3.5 was used [62].

2.3.7 Objective 2: Assess sperm whale diet using isotopic mixing models

Dietary mixing models

To estimate proportional contributions of prey items to sperm whale diets, isotopic mixing

models were used and implemented in the R packages MixSIAR (Bayesian Mixing Stable Isotope Analysis in R) and SIMMR (Stable Isotope Mixing Models in R) [38,63,64]. These models use Bayesian methods to account for uncertainty in input parameters, such as source isotope ratios and trophic enrichment factors [37,38,64]. Isospace plots were used to examine how well the sperm whale samples (predator mixtures) fell within the isotopic space of the prey. Posterior probabilities were estimated using three chains of length 100,000 after a burn-in of 50,000 iterations and chains were thinned by subsampling every 50^{th} iteration. All statistical analyses were performed using the free software package R v.3.5.1 [65].

Models were run for all whales combined and for selected subsets of whales to test the following specific hypotheses:

Hypothesis 1: Sperm whale diet composition did not change over the past 15 years
All whale samples were collected between 2003 and 2017 and were divided into "older" samples collected more than 10 years ago (2003-2009), and "recent" samples (2010-2017) to assess how dietary proportions may have changed over the past 15 years.

Hypothesis 2: Sperm whale diet composition did not exhibit within-season changes
The field season each year ran from May to September, roughly considered "summer", and samples were divided into early, mid, and late summertime periods to assess potential within-season variability in diet.

Hypothesis 3: Sperm whale diet composition was the same between frequent and non-frequent depredators
There is evidence that some whales in the SEASWAP catalog are sighted more frequently than other whales. Of the 122 individual whales in the catalog, 12 individuals make up one-third of all sightings and are referred to as "frequent" depredators. Of the biopsy samples used in this analysis, 10 came from frequent depredators, while the remaining 23 were from non-frequent depredators. We assessed differences in dietary proportions between frequent depredators and non-frequent depredators.

2.4 Results

Thirty-three biopsy samples from sperm whales were archived by SEASWAP between 2003 and 2017 that had sufficient skin to include in stable isotope analysis. With the exception of ragfish (n = 3) and clubhook squid (n = 10), sample sizes for prey ranged from 38 to 52 samples (Table 2.1). The clubhook squid were donated by fishermen and were caught on longline gear, with 10 samples being donated between 2014 and 2017 (Table 2.2). Ragfish were requested from all NMFS surveys (NMFS Longline survey and NMFS Bottom Trawl survey) as well as from the longline fleet members, but only three samples were caught between 2016-2017, all from water depths ≤ 50 m, which is shallower than known sperm whale habitat, and in a region outside of the study area.

2.4.1 Objective 1: Describe isotopic variability and calculate trophic position

Mean stable isotope ratios of sperm whales ranged widely from -20‰ to -17‰ for $\delta^{13}C$ and from 12‰ to 17‰ for $\delta^{15}N$ (Figure 2.2). $\delta^{15}N$ values of sperm whales ranged from 15.2‰ to 18.3‰ ($\bar{x} \pm s.d. = 17.0 \pm 0.7$), resulting in an estimated trophic level of 5.7 ± 0.4 (Table 2.2, Figure 2.2). The estimated mean trophic levels of prey ranged from 3.5 for magister squid to 5.7 for clubhook squid, which fed almost at the same trophic level as sperm whales (Table 2.2, Figure 2.2). Isotopic ratios of the two skate groups (longnose skate vs. "other" skates) were not significantly different (ANCOVA: $p > 0.14$; Table 2.3). Similarly, there were no significant differences in either $\delta^{13}C$ or $\delta^{15}N$ between Pacific grenadier and giant grenadier (ANCOVA: $p > 0.16$; Table 2.3). Therefore, all skate species and both grenadier species were grouped as "skates" and "grenadier", respectively. The $\delta^{13}C$ and $\delta^{15}N$ of magister squid and the $\delta^{13}C$ values of spiny dogfish differed significantly among depth strata (Table 2.3). Nevertheless, because isotope ratios of each depth strata overlapped so closely, and all depth strata were within sperm whale habitat, we considered all specimens of a given species to be representative of the available prey and combined them into a single group across depth strata for use in mixing models. This was also necessary to reduce the number of end members, and thus uncertainty in the model. Similarly, $\delta^{13}C$ values of grenadier increased significantly with length (ANCOVA: $F_{30,38} = 23.49$, $p < 0.001$), but all grenadier were combined into a single group for the mixing model because they had a narrow length range (25 – 40 cm for pre-anal fin length), were found

in the same habitat, and were assumed to be representative of grenadier that sperm whales might consume. Finally, $\delta^{15}N$ values of magister squid increased significantly with length (ANCOVA; $F_{36,42} = 63.30$, $p < 0.001$). All length classes were again grouped for the reasons noted above. In addition, isotope ratios were more similar among conspecifics than among species and were thus naturally grouped by species.

2.4.2 Objective 2: Assess sperm whale diet using isotopic mixing models

Sablefish and spiny dogfish had very similar isotopic ratios (Figure 2.2, Table 2.2), indicating they likely occupy a similar trophic niche space and would be difficult to differentiate in mixing models. Consequently, we grouped these two species together for dietary mixing model analysis. We also ran models with the two species separated, and patterns of dietary proportions between prey items did not change, but those for dogfish and sablefish increased when they were combined (see Appendix 2.3).

Isotope ratios of predator and prey samples plotted together in an "iso-space plot", generally showed sperm whale samples (mixtures) to lie within the mixing polygon defined by the mean of the sources, indicating the data were acceptable for running the mixing models (Figure 2.3) [37]. Five mixtures branched off the main cluster with decreased $\delta^{13}C$ and $\delta^{15}N$ values (Figure 2.3), however they did not come from a specific year, month, or region that would separate them out from the other mixtures in this analysis.

Mixing models for all sperm whale samples and whale groupings showed the sablefish/dogfish group, skates, and rockfish to be the major dietary contributors (Table 2.4). The estimated proportions were highly variable, reflecting variability in diet composition as well as a high degree of uncertainty. The sablefish/dogfish group and skates were the largest contributors to all whale diets in general (mean ± sd: 35.6% ± 13.9% and 25.4% ± 8.3% respectively). All mixing model results suggest that grenadier, clubhook squid, and magister squid generally made up a smaller proportion of sperm whale diets, with each species contributing less than 10% to diets of all whale groups except magister squid in the mid-summer whale group (12.7% ± 10.2%, Table 2.4).

Hypothesis 1: Sperm whale diet composition did not change over the past 15 years

The results of mixing models show that the proportion of sablefish/dogfish in diets was higher in recent samples (58.1% ± 22.5%) than older samples (34.7% ± 13.3%). Similarly, the proportion of shortraker rockfish increased from older samples (9.9% ± 4.7%) to more recent samples (21.5% ± 20.3%) (Table 2.4, Figure 2.4). Model outputs were used to compute the probability that more recently sampled whales had a higher proportion of sablefish/dogfish in their diets than older samples, with a probability of 86%.

In exploring isotopic niche of sampled whales over time, a multivariate analysis of variance (MANOVA) indicated significant differences in $\delta^{13}C$ and $\delta^{15}N$ values among years ($F_{16,48}$=3.23, p=0.001). Subsequent univariate tests for each isotope ratio showed $\delta^{13}C$ values to vary significantly at the 5% level (F=2.98, p=0.02), while $\delta^{15}N$ values did not vary significantly by year (F=2.06, p=0.08).

Hypothesis 2: Sperm whale diet composition did not exhibit seasonal changes

Seasonal mixing model results showed the contribution of sablefish/dogfish increased seasonally from 25.8% ± 20.2% in early summer samples to 43.9% ± 19.5% in late summer samples. Skates contributed the largest proportion to early summer sperm whale diets (30.6% ± 20.3%) (Table 2.4, Figure 2.4).

Hypothesis 3: Sperm whale diet composition was the same between frequent and non-frequent depredators

When frequent and non-frequent depredator groups were compared in mixing models, the diets of frequent depredators were dominated by skates (46.0% ± 20.4%), while non-frequent depredator diets were dominated by the sablefish/dogfish group (48.5% ± 22.3%) (Table 2.4, Figure 2.3). From model outputs, the probability that the proportion of skates in the frequent depredator diets was higher than that of non-frequent depredator diets was 82%. Similarly, the probability that the proportion of sablefish/dogfish in the non-frequent depredator diets was higher than that of the frequent depredator diets was 84%.

2.5 Discussion

2.5.1 Trophic level

This work highlights trophic connections among sperm whales, groundfish, and squid in the GOA, and identifies important prey species that contribute to sperm whale diets in this region. Sperm whales are a top predator in this ecosystem (TL=5.7), closely followed by clubhook squid (TL=5.7), while magister squid had the lowest trophic level estimates (3.5, Table 2.2). Grenadier had $\delta^{13}C$ values more negative than expected, given they generally inhabit the same areas as most other groundfish species sampled (Figure 2.2). This could indicate they spend more time farther offshore and/or foraging in more pelagic habitat than the other species. While there were not significant differences in $\delta^{15}N$ values or TL calculations among years (ANOVAs, F=2.06, p=0.08), there was evidence for significant variability in isotope ratios overall (MANOVA, $F_{16,48}$=3.23, p=0.001), which was driven by $\delta^{13}C$ values (ANOVA, F=2.98, p=0.02). Sample size for each year varied from 1 sample in 2003 and 2015, to 11 samples in 2016, with an average of 3.7 samples per year. With such a low sample size, these results should be taken cautiously, and it is difficult to determine whether sperm whales have changed their diet over the duration of the study. While nothing can be done about low sample sizes in previous years, future work should focus on increasing sample size each year to examine annual patterns in isotope ratios.

2.5.2 Sperm whale diet

In general, our mixing model results suggest that sperm whales sampled in this study area primarily consumed sablefish/dogfish, skates, and shortraker rockfish (Table 2.4). The apparent prevalence of skates, rockfish, and dogfish is consistent with historical stomach contents data from commercial whaling in the mid 1900s, but the notable lack of ragfish and the addition of sablefish distinguishes more recent diets. Whales sampled in the early 2000's had a smaller proportion of the sablefish/dogfish group in their diets than whales sampled more recently between 2009 and 2017, indicating a potential increase in sablefish in diets over time. This complements fishermen's observations that depredation has been increasing over time. MANOVAs indicated year was a significant predictor in $\delta^{13}C$ values, though we must note the small sample sizes of one to six samples in all but one year which could cause individual

variability to bias the results of these tests. Nevertheless, our results indicate there does seem to be fluctuation in the isotope ratios, and potentially diet, of whales across years. Larger sample sizes in future years would help tease out these relationships.

Whales appear to increase sablefish/dogfish consumption throughout the summer fishing season. We note that inferring the time period of prey consumption that is reflected in a given sample is still highly uncertain for whales. Various studies on isotopic incorporation rates of blue whales and bottlenose dolphins have estimated full turnover of skin tissue between 163 ± 91 and 180 ± 71 days respectively for $\delta^{15}N$ values [58,60], which would result in the inner layer of skin representing diet from up to approximately 60 days prior to sampling [44]. If we apply this rate to our sperm whale samples, the early summer samples (mid-May through June) would represent sperm whale diet from mid-March to May, which aligns with the typical mid-March opening of the commercial longline fishing season each year. Conversely, the late-summer samples collected from mid-August through September likely represent diet from mid-June through August. Therefore, these results indicate that the proportion of sablefish in sperm whale diets increases from the start of the longline fishing season through the summer.

The most surprising results from this study were that whales that are sighted most frequently in the SEASWAP catalog consumed a higher proportion of skates than any other prey, while non-frequently sighted depredators had a larger proportion of the sablefish/dogfish group in their diets (Table 2.4, Figure 2.4). While our sample size for the frequent depredators was low (n=10), it is difficult to explain these findings. Biomass estimates for skates are similar to spiny dogfish, at approximately 50,000 t [66], which is much lower than sablefish biomass estimates (~ 488,000 t) [67]. Further, the bycatch of skates is typically higher on halibut fishing sets than sablefish sets, indicating they are found at shallower depths than sablefish. Catch data corroborate that skates on average inhabit shallower depths than sablefish, though their habitats do overlap [66]. Therefore, while skates are bycaught on sablefish fishing gear, they are unlikely to be more available to frequently depredating sperm whales than sablefish. Calorimetry analysis of the lipid content available in each of these species may shed additional light on the values of skates to sperm whales but was outside the scope of this study.

One potential explanation for the high proportion of skates in frequent depredator diets is that a diet item was missing in the analysis that occupies the same isotopic space as skates. However, we are confident that we sampled the major available prey species in the habitat where sperm whales are foraging, especially depredating sperm whales. Species we did not sample were other species of rockfish, such as shortspine thornyheads (*Sebastolobus alascanus*), which are another large bycatch fish in longline fisheries, and Pacific ocean perch (*Sebastes alutus*), both of which were occasionally noted in sperm whale stomachs, though rarely [8,13]. While specific isotopic estimates for these species in GOA waters do not exist, trophic level estimates indicate that isotope ratios are likely very similar to the shortraker rockfish we sampled [68]. Therefore, it is unlikely either of these species occupy the same isotopic space as skates and were therefore an important diet item that was missed in this analysis.

Another explanation for the high proportion of skates in the diets of frequent depredators is that these animals actually prefer skates, and the frequency with which they follow longline fishing vessels brings them to shallower waters where fishermen often deploy "combo sets". These combo sets target both halibut and sablefish. On these shallower sets, skates may be relatively more abundant and may be targeted in addition to sablefish by frequent depredators. Additionally, it is possible that while frequent depredators are targeting sablefish and skates on longline gear while depredating, they may also target skates while naturally foraging. Indeed stomach contents data from sperm whales killed off central California shows that after clubhook squid, longnose skates were the second highest diet item [69].

The frequent depredator samples were collected across the summer months (early, mid, and late summer samples), though two-thirds of them were collected during July, which would reflect diet of whales back to May. A few fishermen have noted that in certain areas of the GOA they catch a lot of skate egg sacks on their longline gear while fishing for sablefish in May and early June. This may suggest that skates move into deeper water nurseries in early summer, where depredation and whale activity is more prevalent. Indeed there were reports of skate eggs found in sperm whale stomachs during commercial whaling, though the report came from the western North Pacific, near Olyotorsky Bay on the Russian coast [9]. In our study, early season diets of frequent depredators were based on six samples that may be driving the apparent skate

preference if these whales consumed a large proportion of skates while foraging and depredating around skate nurseries at that time of year.

While our plots generally showed prey covering our mixtures (sperm whale samples) sufficiently, there were a small number of mixtures that were slightly outside of the prey isospace when drawing a polygon around source means (Figure 2.3) [37]. This is an indication that a potential prey item could have been missing, or trophic enrichment factors could be under-estimated. Our trophic enrichment factors were estimated from literature values, and models were run with both a low and high estimate from marine mammal studies (Appendix 2.4). While the low and high TEFs resulted in slightly different isospace plots, general patterns were the same, and the same group of mixtures remained outside of the prey space. The other potential prey items from their GOA foraging grounds such as Pacific halibut or other rockfish species likely don't occupy isotopic space where the outlying mixtures were located, as discussed above. Some fishermen have wondered if sperm whales eat Pacific sleeper shark (*Somniosus pacificus*) in the region, though no historic evidence exists to support this theory, nor were we able to find isotope values to include in or compare to our study.

An alternative explanation for small differences between mixtures and prey in isospace plots is that there were prey items consumed by sperm whales outside of Alaskan waters. The samples that fall near the outer edges of the sampled prey in Figure 2.3 could have come from whales that had recently arrived in the GOA and were feeding on other species on their way north. Humboldt squid (*Dosidicus gigas*) are a preferred prey item in warmer equatorial waters off Mexico, and Central America [33,70]. SEASWAP has tracked a number of sperm whales using satellite tags, which have made broad-scale movements from GOA waters to Mexican waters [71]. Isotope studies that have been done in these areas have found delta values of *D. gigas* consistent with where the outlier mixtures were located in the isospace plots, after applying TEF estimates (Figure 2.3) [33,70]. Recent range expansions of *D. gigas* into Northeast Pacific waters further suggests the GOA sperm whales sampled in this study may have been feeding on these species as they moved toward the GOA [72].

2.5.3 Caveats

While ragfish were listed as an important diet item to sperm whales in our study area during historical commercial whaling, we were unable to collect any specimens in our study area during this study. This could indicate that ragfish are less abundant than they were historically or that they primarily occupy a different habitat than those we were able to sample through surveys and opportunistic fishery donations, such as higher in the water column or at deeper depths. Indeed, there is some evidence from descriptions of ragfish that they are adapted to inhabit deep depths [14]. It is also possible they are not vulnerable to the gear used to collect samples (bottom trawl and demersal longline gear). From an isotopic perspective, if they lived much deeper, or in a different place in the water column, we would expect their isotope ratios to reflect that different habitat [68,73–76]. However, the isotopic composition of the three specimens we were able to acquire to the northwest of our study region were similar to those of spiny dogfish, indicating they likely inhabit a similar depth range or have similar prey preferences (Appendix 2.2).

The isotopic similarities between sablefish and dogfish were unfortunate in that sablefish were the species of interest in this study. Mixing models would be unable to differentiate between the two sources, and thus they were grouped together. Both species are demersal fish as adults, found in the same general habitat of the continental slope, and stomach contents research has shown a majority of their diets to both be from fishery offal, though their diets do differ slightly between crustaceans and fishes [77,78]. This suggests they may occupy different trophic niches, though similar isotopic niches. We believe that whales feeding in the isotopic space of these two sources are likely consuming much larger proportions of sablefish than dogfish for several reasons. First, recent sablefish biomass estimates (~488,000 t) [67] are more than triple that of spiny dogfish (55,000 t) [79] in the GOA, indicating they are likely more biologically available. Second, sablefish are targeted by sperm whales in depredation activity [6,17,18]. Therefore, while the group may include some spiny dogfish, a majority of the group is likely comprised of sablefish. Future analyses using fatty acid signatures of prey and sperm whale tissue may be able to estimate the proportions of sablefish and dogfish in sperm whale diets.

It is important to note that this study was done using data that was often collected opportunistically during other projects. As such, samples were collected only during summer

months (May through September), and sometimes only during a single month or week of a given year. No samples were collected outside of longline fishing season during the winter months, when sperm whales may be naturally foraging in GOA waters. Peak effort for longline fishing typically happens in the first few months of the fishery in March and April, and fishermen often try to fish as early in the season as possible when they believe there are fewer sperm whales on the GOA fishing grounds. This likely has an impact on how depredation is spread out throughout the fishery, and how prey preferences and dietary contributions shift throughout the season. Future work to solidify isotopic incorporation rates, coupled with more dedicated sampling effort through all seasons of the year, would help elucidate some of the remaining questions regarding sperm whale prey preferences in the GOA and how they are changing over time.

2.5.4 Summary and next steps

This work represents an important first step in describing the foraging ecology of sperm whales and their prey in the GOA. While some work has been done to explore diving behavior and how whales interact acoustically with longline fishing gear in this region [25], this project highlights patterns in the contribution of various prey to sperm whale diets. These findings have implications on management of groundfish stocks and provide specific insights to managers on the commercially valuable sablefish stock. The lack of sablefish as sperm whale diet in historical stomach contents records from commercial whaling suggests that consuming sablefish is relatively new for sperm whales and that depredation reflects a new source of mortality that sablefish did not experience in the past. Hence this mortality should be accounted for in the stock assessment, separately from other natural mortality. While sablefish were not an important prey item historically, they currently make up over 50% of the diet for individuals in this region, which is due at least in part to depredation activity. This apparent continued increase in the proportion of sablefish in diets is noteworthy and is consistent with looking at the same issue from the NMFS survey and fishermen perspectives, which have also shown a potential increase in depredation-related mortality over time and a behavioral spreading of depredation [21,80,81]. Our work suggests that sperm whales have not only changed behaviors to target sablefish on longlines, but that they actually switched their prey in response to discovering the easy availability of the lipid-rich sablefish food source. Future work to compare energy content of all prey items in this study may inform whether or not whales have switched to a better prey

resource in sablefish, and more importantly speak to ecosystem functions and how changes in trophic pathways may be impacting energy flow and ecosystem functions.

While the recovery of whales after the secession of commercial whaling is often cited as an increase in resource conflicts throughout the world, this work shows that at least for sperm whales, the conflict could simply have arisen from whales adopting a new diet in response to an opportunity, regardless of the changes in the number of sperm whales. Working with the commercial fleet and managers together through collaborative research projects will be an integral part of further understanding how to manage sperm whale interactions with both fisheries and the primary prey species (sablefish, skates, and rockfish) identified in this project.

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2.9 Figures

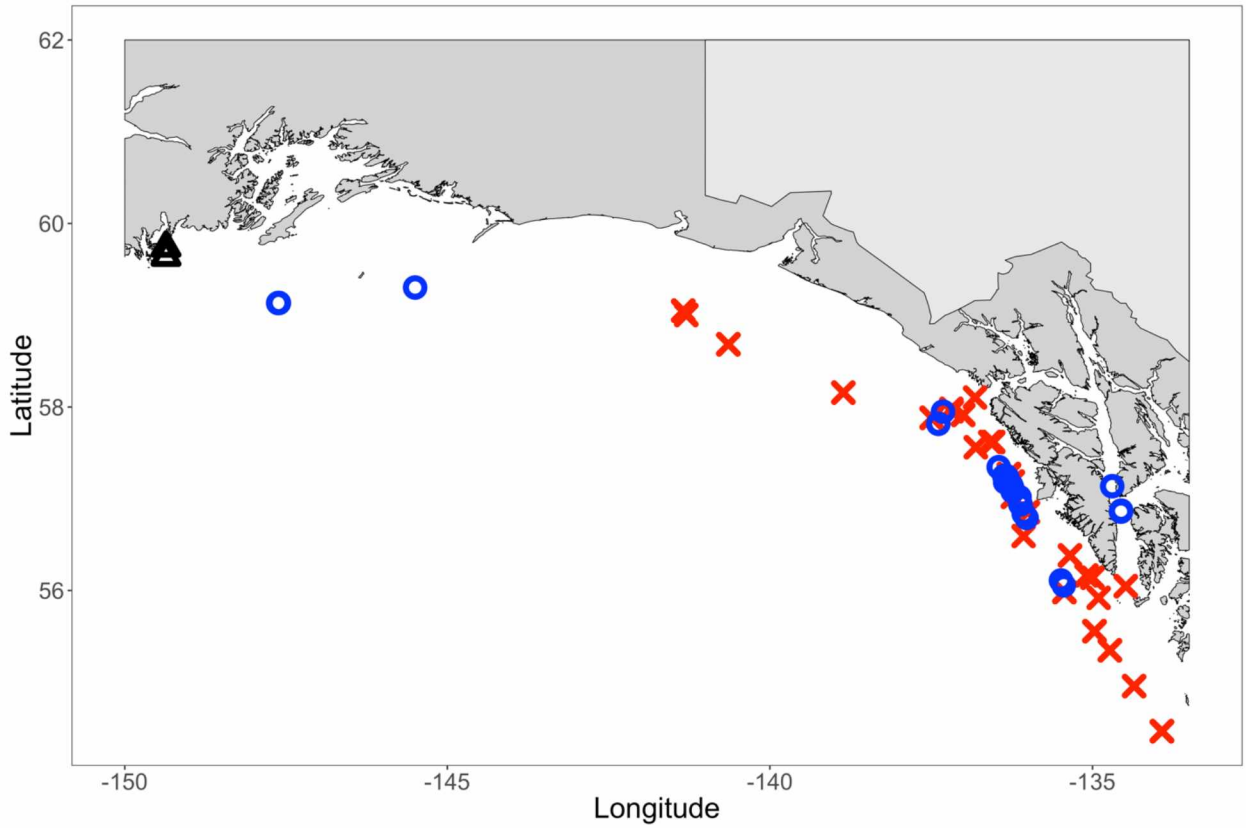

Figure 2.1 Map of the Gulf of Alaska showing locations where biopsy samples (blue circles) and prey (red x's) were collected. The two prey locations (black triangles) in the central GOA represent the locations of the three ragfish collected, all outside of the study area.

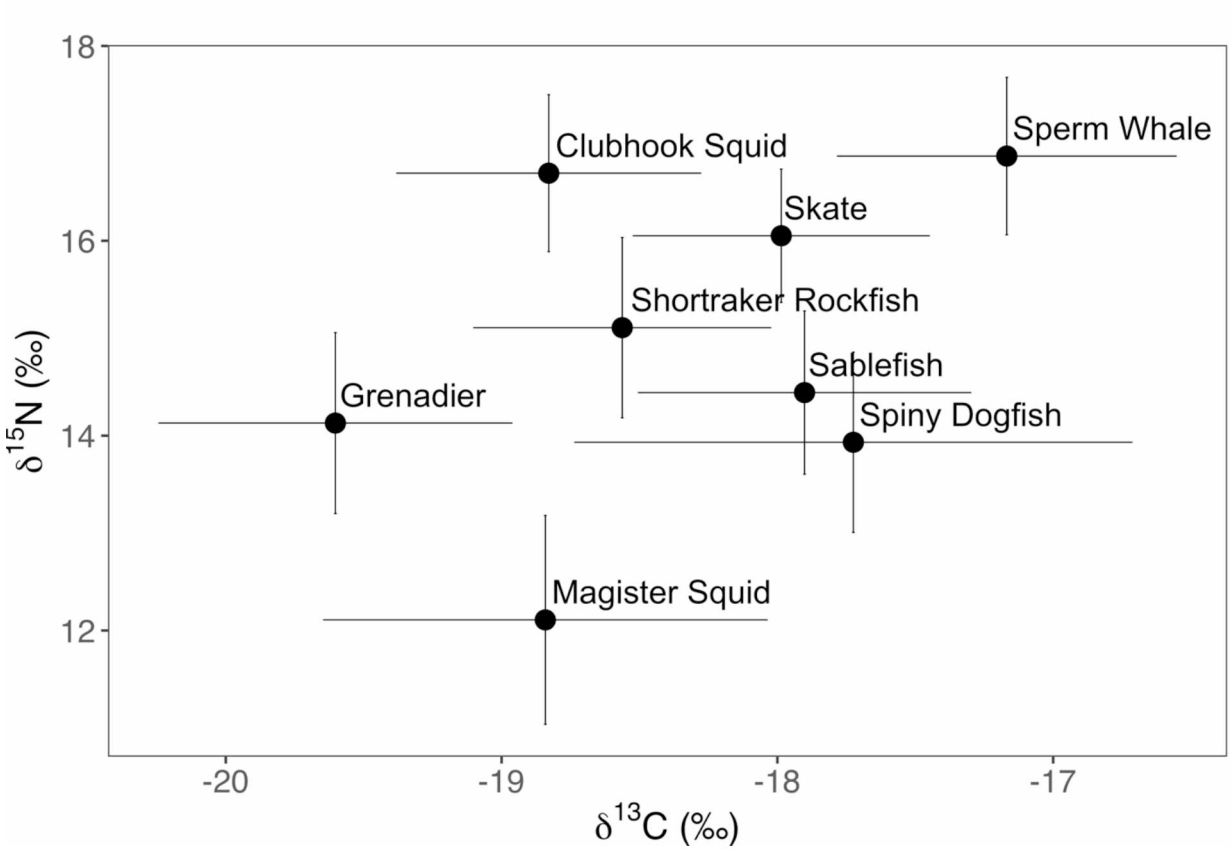

Figure 2.2 Stable isotope ratios of sperm whales and presumed prey items in the Gulf of Alaska. Points are means for each species, while error bars represent one standard deviation from the mean.

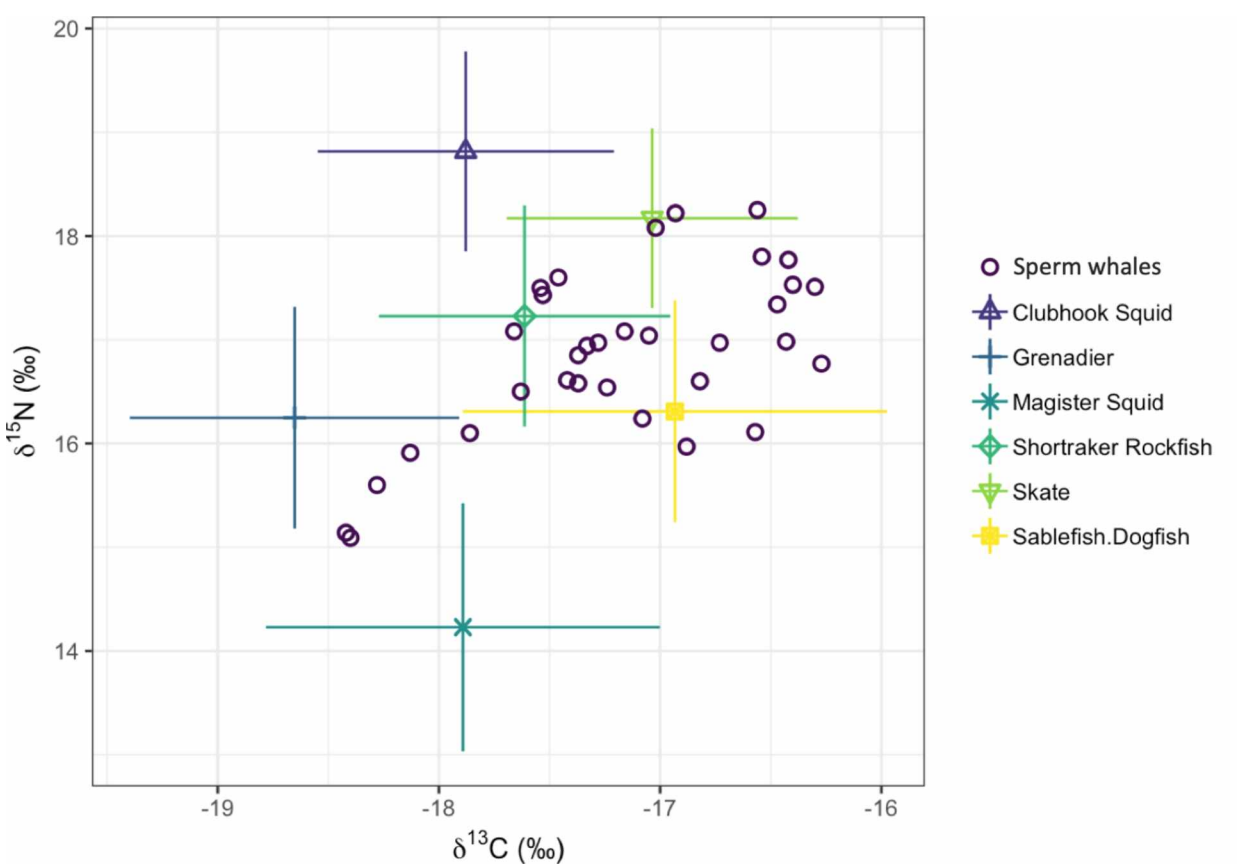

Figure 2.3 Isospace plot showing how sperm whale samples ("mixtures") fit within prey space after trophic enrichment factors have been applied to prey. Error bars indicate combined source and discrimination uncertainty ± 1 sd.

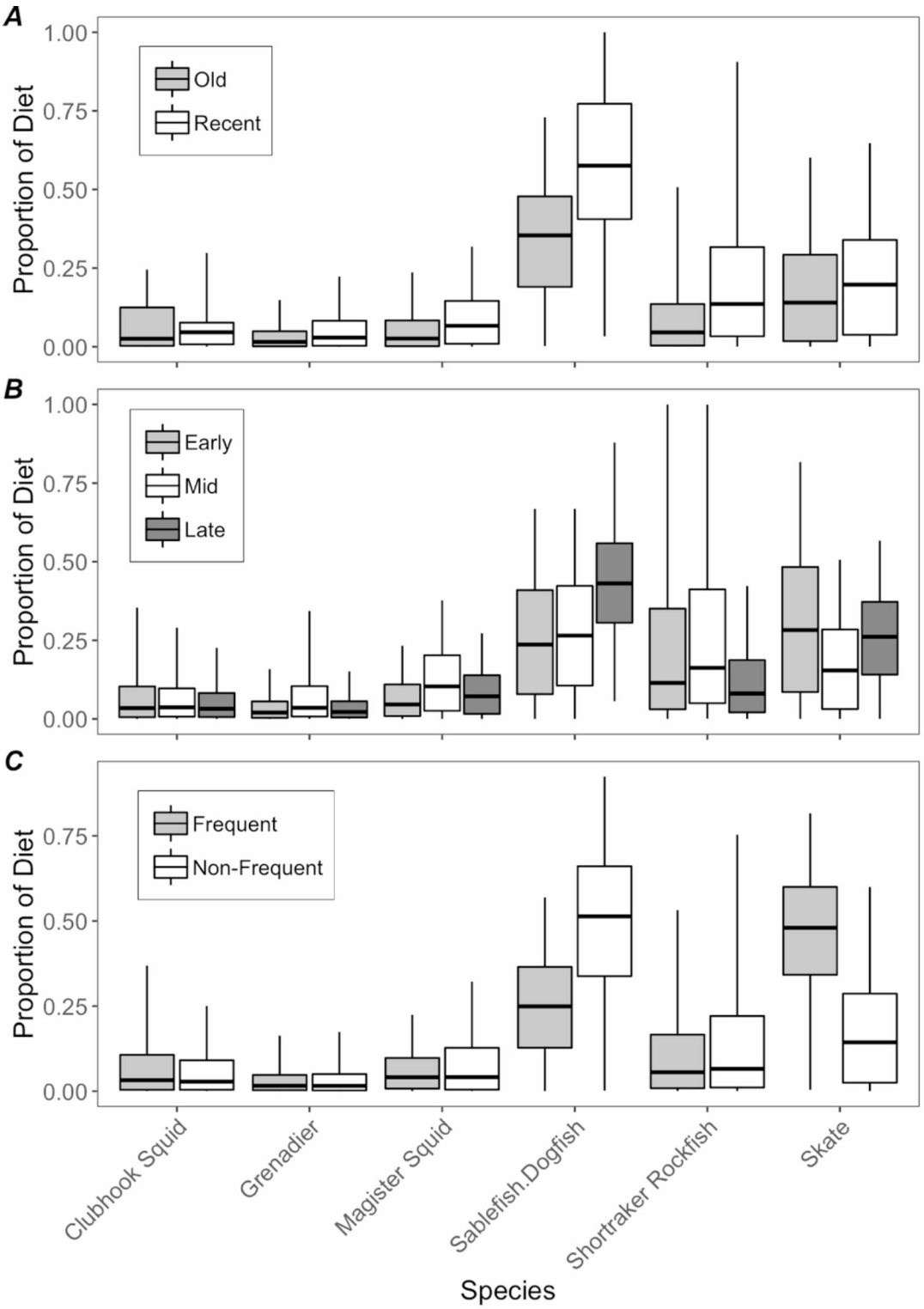

Figure 2.4 Boxplots showing mixing model estimates of the proportional contribution of each prey to sperm whale diets to compare (A) older versus more recent samples; (B) frequent versus non-frequent depredators; and (C) early, mid, and late summer samples. Boxes represent lower and upper quartiles with a median line, while ends of whiskers show 95% credible intervals.

2.10 Tables

Table 2.1 Description of samples collected for this study: whether they were in historical stomach contents, collection year, gear type (BT=bottom trawl, LL=longline), and sample size.

Species	**Historical diet?**	**Year**	**Gear**	***n***
Predator				
Sperm Whale	-	2003-2017	Biopsy	33
Prey				
Ragfish[a]	Yes	2017	BT[b]	3
Shortraker rockfish	Yes	2016-17	LL[b]	45
Skate	Yes	2016-17	LL[b]	42
Spiny dogfish	Yes	2016-17	LL[b]	34
Clubhook squid	Yes	2014-17	LL[c]	10
Magister squid	Yes	2016-17	BT[b]/LL[c]	42
Sablefish	No	2016-17	LL[b,c]	45
Grenadier	No	2016-17	LL[b]	44
Baseline				
Neocalanus spp.	-	2016-17	Bongo	62

[a]Collected outside study area; [b]Collected on NMFS survey gear; [c]Collected from commercial fishermen;

Table 2.2 Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ values) and trophic level calculations of sperm whales and each of their potential prey items, ordered by trophic level.

	n	$\delta^{15}N \pm$ s.d. (‰)	$\delta^{13}C \pm$ s.d. (‰)	Trophic Level
Predator				
Sperm whale	33	16.9 ± 0.8	-17.2 ± 0.6	5.7 ± 0.4
Prey				
Clubhook squid	10	16.7 ± 0.8	-18.8 ± 0.6	5.7 ± 0.4
Skate	45	16.1 ± 0.7	-17.9 ± 0.5	5.4 ± 0.3
Shortraker rockfish	45	15.1 ± 0.9	-18.6 ± 0.5	4.9 ± 0.4
Sablefish	45	14.4 ± 0.8	-17.9 ± 0.6	4.6 ± 0.4
Grenadier	45	14.1 ± 0.9	-19.6 ± 0.6	4.5 ± 0.4
Spiny dogfish	38	13.9 ± 0.9	-17.8 ± 0.8	4.4 ± 0.4
Magister squid	45	12.1 ± 1.1	-18.8 ± 0.8	3.5 ± 0.2
Baseline				
Neocalanus spp.	62	8.9 ± 0.5	-19.2 ± 0.9	2.0

Table 2.3 Analysis of Co-Variance (ANCOVA) results for each species and isotope relationships with length and depth. For skates and grenadier, species level relationships were tested as well. Results in bold are those that were significant. Clubhook squid depth strata and lengths were not consistently or accurately recorded by commercial fishermen so they were not included.

Species	**Factor**	**$\delta^{13}C$**		**$\delta^{15}N$**	
		F-value	***p*-value**	**F-value**	***p*-value**
Clubhook squid	Length				
	Depth.Strata				
Skate	Length	**$F_{42,43}$=34.90**	**0.001**	$F_{42,43}$=3.12	0.128
	Depth.Strata	$F_{5,43}$=5.15	0.052	$F_{5,43}$=5.25	0.048
	Sub.Species	$F_{1,43}$=0.23	0.648	$F_{1,43}$=2.78	0.146
Shortraker rockfish	Length	$F_{25,38}$=0.05	0.817	$F_{25,38}$=0.58	0.449
	Depth.Strata	$F_{5,38}$=0.71	0.556	$F_{5,38}$=1.29	0.291
Sablefish	Length	$F_{26,32}$=0.78	0.385	$F_{26,32}$=3.85	0.058
	Depth.Strata	$F_{5,32}$=1.21	0.326	$F_{5,32}$=0.44	0.816
Grenadier	Length	**$F_{30,38}$=23.49**	**<0.001**	$F_{30,38}$=3.65	0.064
	Depth.Strata	$F_{5,38}$=0.10	0.961	$F_{5,38}$=0.38	0.767
	Sub.Species	$F_{1,38}$=2.01	0.164	$F_{1,38}$=1.55	0.221
Spiny dogfish	Length	$F_{26,32}$=0.81	0.375	$F_{26,32}$=4.62	0.041
	Depth.Strata	**$F_{5,32}$=4.84**	**0.007**	$F_{5,32}$=1.58	0.214
Magister squid	Length	$F_{36,42}$=3.61	0.072	**$F_{36,42}$=63.3**	**<0.001**
	Depth.Strata	**$F_{5,42}$=9.21**	**<0.001**	**$F_{5,42}$=6.40**	**<0.001**

Table 2.4 Summary of estimated contributions (mean ± sd) of each prey item to sperm whale diets. Whales are grouped by all whales, older/recent samples, early/mid/late summer samples, and frequent/non-frequent depredators.

		Temporal		Seasonal			Depredation Activity	
Species	**All**	**Old**	**Recent**	**Early**	**Mid**	**Late**	**Frequent**	**Non-Frequent**
Clubhook Squid	8.3 ± 6.6	5.4 ± 6.8	7.8 ± 7.1	7.2 ± 9.6	6.7 ± 7.2	5.6 ± 6.5	7.6 ± 6.1	5.9 ± 4.3
Skate	25.4 ± 8.3	18.1 ± 7.7	21.5 ± 12.4	30.6 ± 24.3	17.7 ± 10.3	26.2 ± 14.9	46.0 ± 20.4	18.4 ± 15.7
Shortraker Rockfish	14.5 ± 11.1	9.9 ± 4.7	21.5 ± 20.3	25.8 ± 21.9	28.2 ± 21.9	11.9 ± 10.2	11.6 ± 11.5	15.4 ± 12.6
Sablefish.Dogfish	35.6 ± 13.9	34.7 ± 13.3	58.1 ± 22.5	25.8 ± 20.2	27.5 ± 17.8	43.9 ± 19.5	25.1 ± 15.1	48.5 ± 22.3
Grenadier	5.7 ± 4.7	3.3 ± 4.1	5.3 ± 6.3	3.7 ± 4.5	7.2 ± 6.9	3.8 ± 3.4	3.4 ± 4.7	3.6 ± 4.9
Magister Squid	10.6 ± 6.8	5.4 ± 3.8	9.1 ± 7.2	6.7 ± 6.8	12.7 ± 10.2	8.7 ± 7.9	6.2 ± 6.1	8.1 ± 9.5

2.11 Appendices

A2.1 Including ragfish in stable isotope analysis

We attempted to collect ragfish in our experiment due to their prevalence in diets of sperm whales during commercial whaling, but were only able to obtain three samples, all of which were collected outside the study area. When we included ragfish in the isotope analysis, they fell in similar isotopic space as both sablefish and spiny dogfish (Figure A2.1.1). Mixing models would have a difficult time isolating the overlapping prey sources, suggesting the ragfish be combined with sablefish and dogfish prey for analysis.

A2.2 Mixing model results including ragfish in models

When mixing models were run with ragfish included, proportions of prey to sperm whale diets remained similar to results without ragfish (Figure A2.2.1, Table A2.2.1). The sablefish/dogfish group, with ragfish added, remained the highest proportion of sperm whale diets, as well as skates (Figure A2.2.1, Table A2.2.1).

A2.3 Mixing model results separating sablefish and dogfish

Though sablefish and dogfish occupy similar isotopic space and were combined for the analysis, we ran separate mixing models here with sablefish and dogfish listed separately. Even when separated, sablefish and dogfish both had relatively high contribution to sperm whale diets, with skates being nearly as high (Figure A2.3.1, Table A2.2.1). Top contributors to sperm whale diets were sablefish, rockfish, skates, and dogfish (Figure A2.3.1).

A2.4 Mixing model results using low and high trophic enrichment levels

We ran mixing models separately to explore the effect of trophic enrichment factor (TEF) on results. TEFs for $\delta^{15}N$ values in the skin of free ranging pilot whales and fin whales have been estimated at 1.7‰ and 2.8‰ respectively [1,2]. TEFs for $\delta^{13}C$ values from each of these studies remained about 1‰. To explore how TEF estimates affected our results, we ran mixing models with each of these estimates separately. Using a lower TEF notably increased the dietary contribution of skates to sperm whale diets, and to a lesser extent clubhook squid (Figure A2.4.1, Table A2.2.1). Using a higher estimate of TEF notably increased the dietary contribution of

magister squid to sperm whale diets (Figure A2.4.2, Table A2.2.1). Under both the low and high TEF estimates, the sablefish/dogfish group and rockfish remained higher contributors to sperm whale diet as well (Figures A2.4.1-2, Table A2.2.1).

A2.5 References

1. Borrell A, Abad-Oliva N, Gõmez-Campos E, Giménez J, Aguilar A. 2012 Discrimination of stable isotopes in fin whale tissues and application to diet assessment in cetaceans. *Rapid Commun. Mass Spectrom.* **26**, 1596–1602. (doi:10.1002/rcm.6267)
2. Abend AG, Smith TD. 1997 Differences in stable isotope ratios of carbon and nitrogen between long-finned pilot whales (Globicephala melas) and their primary prey in the western north Atlantic. *ICES J. Mar. Sci.* **54**, 500–503. (doi:10.1006/jmsc.1996.0192)

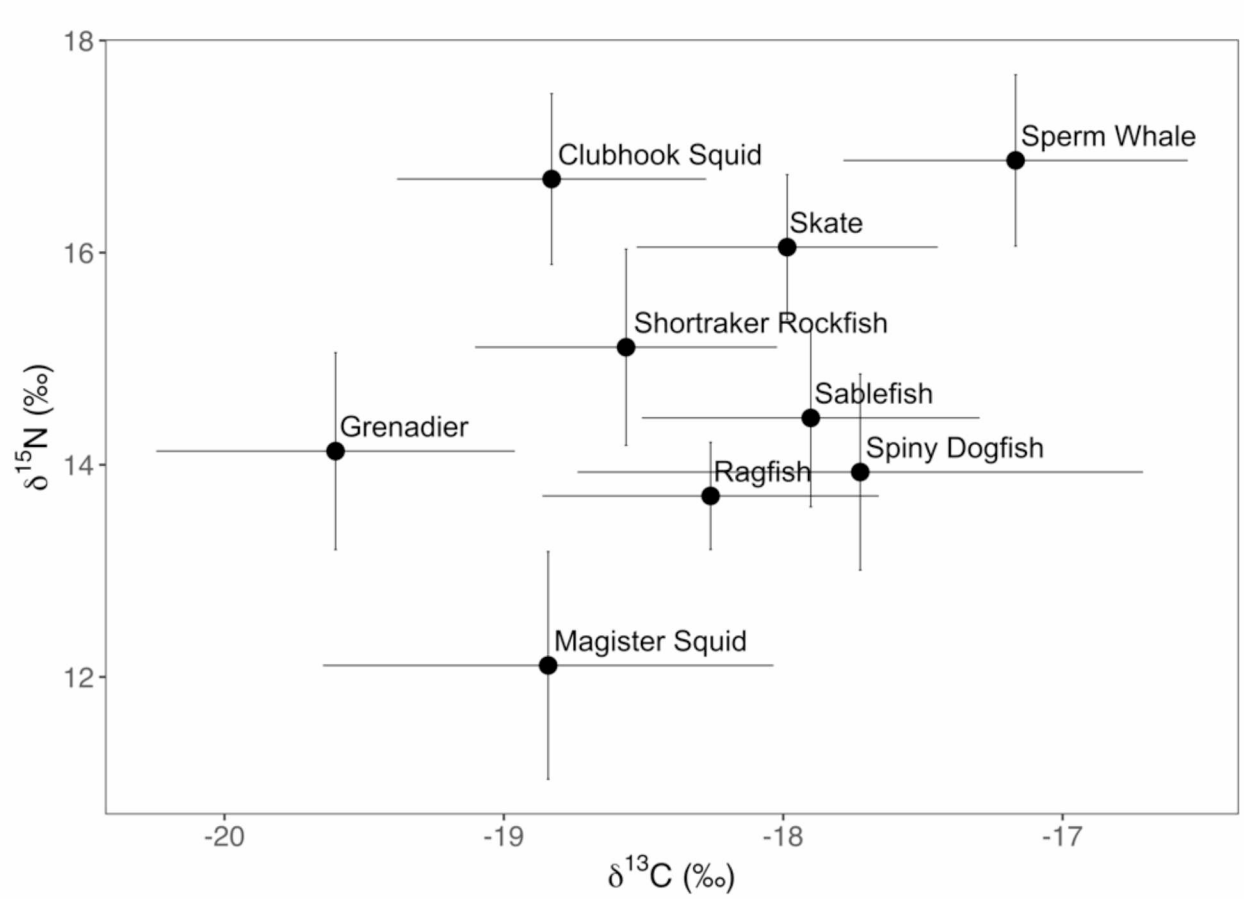

Figure A2.1.1 Stable isotope ratios of sperm whales and presumed prey items, including ragfish, in the Gulf of Alaska. Points are means for each species, while error bars represent one standard deviation from the mean.

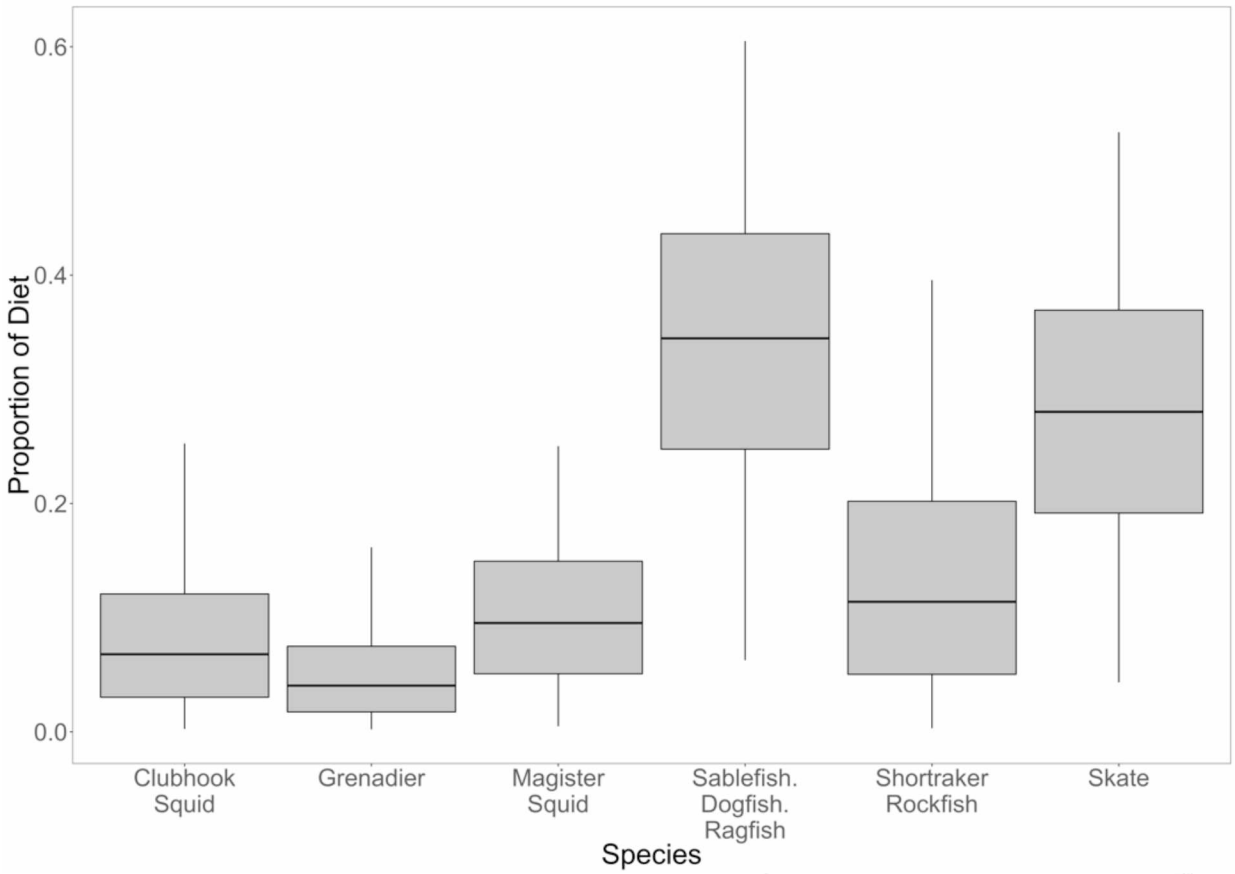

Figure A2.2.1 Boxplots showing mixing model estimates of the proportional contribution of each prey to sperm whale diets, with ragfish included. Boxes represent lower and upper quartiles with a median line, while ends of whiskers show 95% credible intervals.

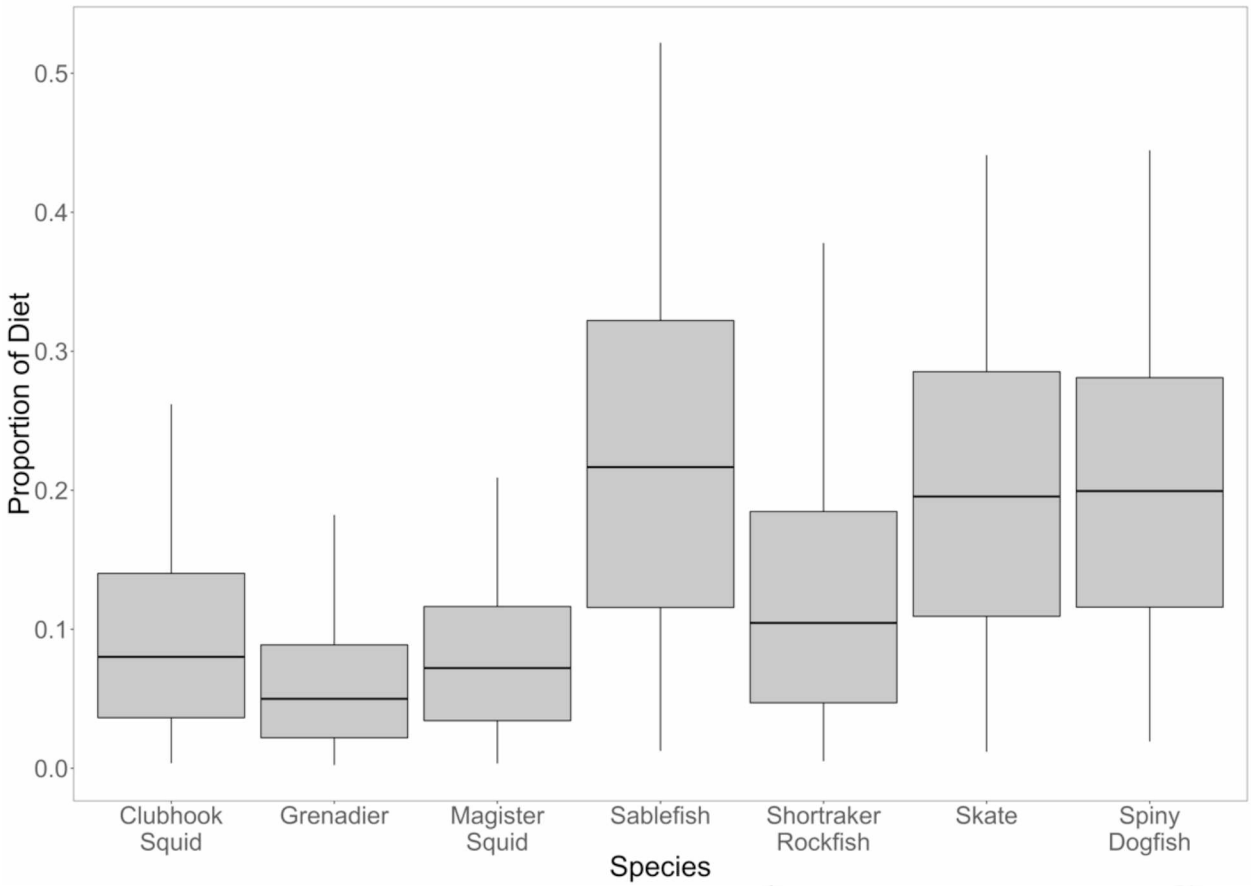

Figure A2.3.1 Boxplots showing mixing model estimates of the proportional contribution of each prey to sperm whale diets, with sablefish and dogfish separated. Boxes represent lower and upper quartiles with a median line, while ends of whiskers show 95% credible intervals.

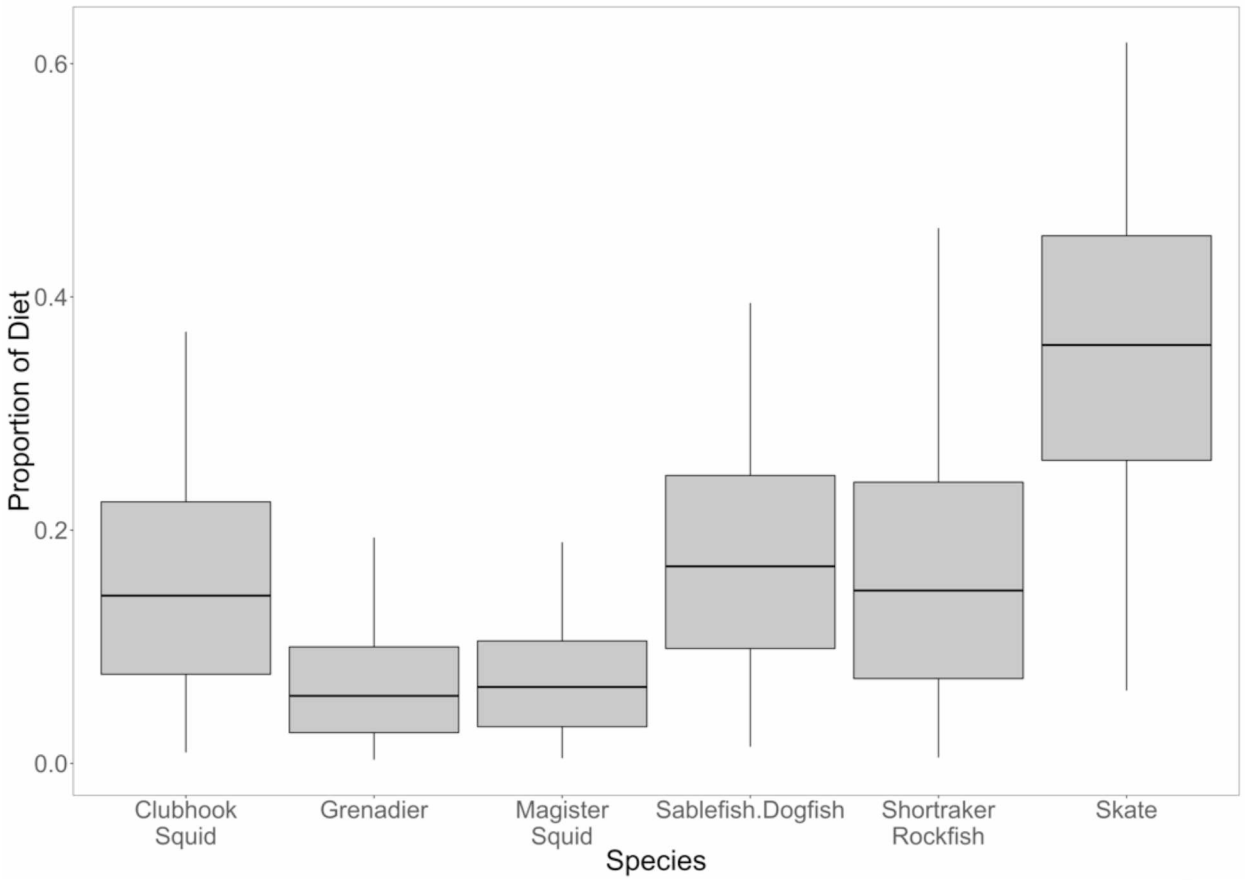

Figure A2.4.1 Boxplots showing mixing model estimates of the proportional contribution of each prey to sperm whale diets, using a low estimate of trophic enrichment factor (TEF) of 1.7‰ for $\delta^{15}N$ values. Boxes represent lower and upper quartiles with a median line, while ends of whiskers show 95% credible intervals.

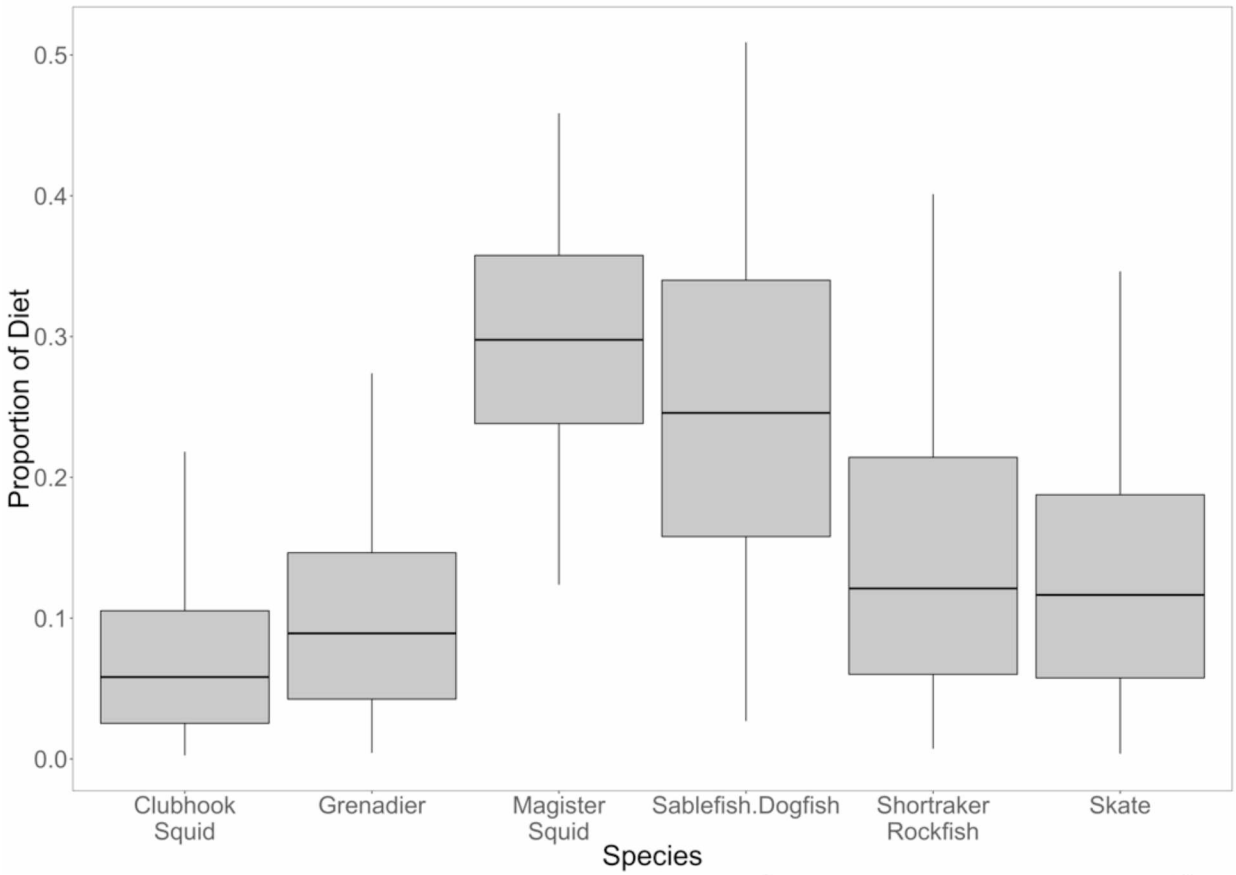

Figure A2.4.2 Boxplots showing mixing model estimates of the proportional contribution of each prey to sperm whale diets, using a high estimate of trophic enrichment factor (TEF) of 2.8‰ for $\delta^{15}N$ values. Boxes represent lower and upper quartiles with a median line, while ends of whiskers show 95% credible intervals.

Table A2.2.1 Summary of estimated contributions (mean ± sd) of each prey item to sperm whale diets. Columns show all prey with sablefish & dogfish combined as seen in the main manuscript, all prey with ragfish added, all prey with sablefish and dogfish separated, all prey with a low end trophic enrichment factor (TEF) applied (Abend and Smith, 1997), and all prey with a high end TEF applied (Borrell *et al.*, 2012).

Species	**Sablefish & Dogfish combined**	**Ragfish added**	**Separated Sablefish & Dogfish**	**Low TEF**	**High TEF**
Clubhook Squid	8.3 ± 6.6	8.4 ± 6.7	9.4 ± 7.1	15.7 ± 10.0	7.3 ± 5.9
Grenadier	5.7 ± 4.7	5.2 ± 4.4	6.1 ± 4.9	6.9 ± 5.3	10.2 ± 7.5
Magister Squid	10.6 ± 6.8	10.4 ± 6.7	8.1 ± 5.7	7.3 ± 5.1	29.6 ± 8.7
Sablefish.Dogfish.Ragfish	-	34.2 ± 13.8	-	-	-
Sablefish.Dogfish	35.6 ± 13.9	-	-	17.8 ± 10.3	25.1 ± 12.7
Sablefish	-	-	22.7 ± 13.9	-	-
Dogfish	-	-	20.5 ± 11.4	-	-
Shortraker Rockfish	14.5 ± 11.1	13.8 ± 10.9	12.9 ± 10.2	16.9 ± 12.3	14.6 ± 10.9
Skate	25.4 ± 8.3	28.1 ± 12.6	20.3 ± 11.6	35.3 ± 14.2	13.2 ± 9.3

Chapter 3 Movement and diving behavior of satellite-tagged male sperm whales in the Gulf of Alaska

3.1 Abstract

Male sperm whales (*Physeter macrocephalus*) are known to interact with and depredate from commercial longline fishing vessels targeting sablefish (*Anoplopoma fimbria*) in the Gulf of Alaska (GOA). However, little is known about their movement patterns and diving behavior in this region, and how it is influenced by depredation. Our goals in this study were to better understand sperm whales' use of space in the GOA and explore their diving behavior. Between 2007 and 2016 a total of 33 satellite tags were deployed on sperm whales interacting with fishing vessels in the eastern GOA. A subset of these tags also collected dive characteristics. Given the sporadic nature and low frequency of satellite tag data from deep diving cetaceans, we used state space models to interpolate hourly positions from tags. Of the full tag data set, 29 tag records were usable in this analysis, 14 of which had associated dive information. Nine of the tagged whales moved south out of the GOA before the tags stopped transmitting, with five of these tags remaining on the whales until they were in California or Mexican waters. Minimum rates of horizontal movement were much lower (1.4 km/hr) in GOA waters than south of the GOA (5.5 km/hr), indicating tagged whales sped up when they left the GOA. Behavioral states indicated primarily foraging behavior (82% of locations) in the GOA and primarily transiting behavior (74% of locations) when whales left the GOA heading south. Dive data showed average (±SD) maximum dive depths of 396 m (±166), and dive durations of 32 min (± 9). A majority of dives were classified as square-shaped (72%), followed by U-shaped (23%) and V-shaped (6%) dives. Generalized additive models indicated that dives were significantly deeper and longer during the daytime than dawn, dusk, or nighttime, and dives were significantly deeper and shorter during quarter moons, when tidal currents are weakest. Maximum dive depth decreased in areas of higher sablefish CPUE, and decreased as seafloor depth increased, up to seafloor depths of 800 m, at which point dives became deeper with increasing seafloor depths. Our results show potential links between diving behavior and depredation behavior, indicate whales are likely diving to target both bathypelagic and mesopelagic prey, and highlight the importance of the GOA continental slope as a foraging ground for these individuals. Overall we provide new

insights into the diving behavior and movement of male sperm whales in a high latitude foraging ground.

3.2 Introduction

Sperm whales (*Physeter macrocephalus*) are a deep diving marine predator whose movement, population dynamics, and stock structure are poorly understood throughout much of their worldwide range. In the United States (US), they are listed as endangered under the Endangered Species Act (ESA) of 1973, and depleted under the Marine Mammal Protection Act (MMPA) of 1972 (Muto *et al.*, 2018). Management of sperm whales in US waters is often hindered by a lack of reliable data on regional stock structure and population dynamics, which is important in establishing recovery plans required by both ESA and MMPA listings. Females, juveniles, and calves are thought to inhabit warmer equatorial waters, while mature males move to higher latitude foraging grounds (Caldwell *et al.*, 1966; Best, 1979; Rice, 1989; Whitehead, 2003), though the movement and timing of movements between these areas are not well studied. In fact, movement of males has been identified as one of the largest knowledge gaps in global understanding of sperm whales (Whitehead, 2003). It is thought that in these high latitude foraging grounds, males are usually found in small bachelor groups or alone (Caldwell *et al.*, 1966; Best, 1979; Reeves *et al.*, 1985; Rice, 1989).

The Gulf of Alaska (GOA) represents a high latitude foraging ground of male sperm whales. In the North Pacific and GOA, sperm whales were heavily exploited during commercial whaling through the 1970's (Mizroch and Rice, 2013; Ivashchenko and Clapham, 2014). The effects of this exploitation on patterns of occurrence and stock structure of sperm whales is not known. There are currently three genetically distinct stocks recognized in the North Pacific: Alaska, California Current, and Hawaii (Mesnick *et al.*, 2011). Genetic analysis has also shown that the males that make up the Alaska stock originate from multiple lower latitude populations (Mesnick *et al.*, 2011). There is acoustic evidence that sperm whales are present year-round in the GOA, though incidences of detections are 70% higher in summer months compared to winter months (Mellinger *et al.*, 2004; Diogou *et al.*, 2019).

In their GOA foraging grounds, sperm whales are known to remove fish from commercial longline fishing gear targeting sablefish (*Anoplopoma fimbria*) (Hill *et al.*, 1999; Sigler *et al.*, 2008). This removal, known as depredation, incurs economic costs to the fishery, increases risk of entanglement for whales, and impacts the NOAA Fisheries annual stock assessment survey (Hill *et al.*, 1999; Sigler *et al.*, 2008; Straley *et al.*, 2015; Peterson and Hanselman, 2017; Hanselman *et al.*, 2018a). Since 2003 the Southeast Alaska Sperm Whale Avoidance Project (SEASWAP) has been studying sperm whale depredation in the GOA, with a goal to minimize interactions (Straley *et al.*, 2015). Using fishing vessels as a platform for research, this collaboration between fishermen, scientists, and managers has been successful in gaining important insights into the interactions of sperm whales with fishing vessels in the GOA. SEASWAP has found that sperm whales engaging in depredation in the GOA are male (Mesnick *et al.*, 2011; Straley *et al.*, 2015), and that they are attracted to the distinct acoustic pattern of propeller cavitation made when longline fishing vessels haul their gear (Thode *et al.*, 2007; Wild *et al.*, 2017). Catch-per-unit-effort (CPUE) data show that fishing sets with higher CPUE were also associated with higher presence of whales, while fishers that experienced low CPUE often experienced no depredation, suggesting that whales know where the good fishing locations are (Straley *et al.*, 2015). It also suggests that sperm whale movements in this region are tied to fishing activity in addition to finding natural prey resources.

Food availability and prey resources are a main contributor to the occurrences, movement and diving behavior of sperm whales throughout their worldwide range (Rice, 1989; Watwood *et al.*, 2006). In general, they primarily forage on bathypelagic and mesopelagic prey, at average depths between 200 m and 1,000 m (Rice, 1989; Watwood et al., 2006; Guerra et al., 2017). Sperm whale prey in much of the world consists primarily of cephalopods, however both stomach contents data from commercial whaling and recent stable isotope analysis indicates that fish are prevalent in male sperm whale diets in the eastern GOA and off the British Columbia coast (Okutani and Nemoto, 1964; Flinn *et al.*, 2002; Nichol *et al.*, 2002; Wild *et al.*, 2020). Additionally, sablefish in particular are known to be targeted by sperm whales in the GOA during depredation. Current sperm whale diets in this region consist mainly of sablefish, spiny dogfish (*Squalus suckleyi*), skates (*Rajidae* sp.), and rockfish (*Sebastes* sp.), with lesser proportions of robust clubhook squid (*Onykia robusta*) and magister armhook squid

(*Berryteuthis magister*) (Wild *et al.*, 2020). Though diet of sperm whales is becoming better understood in their GOA foraging grounds, the movement patterns and diving behavior associated with foraging are still poorly understood.

Foraging behavior and movement of male sperm whales in high latitudes is difficult to observe because of the remoteness, high costs, and difficult weather and oceanic conditions in these regions. Much of the literature focuses on acoustic studies using animal-borne suction-cupped archival tags to assess fine-scale movement and acoustic activity during foraging dives (Madsen *et al.*, 2002a, 2002b; Miller *et al.*, 2004; Watwood *et al.*, 2006; Teloni *et al.*, 2008; Mathias *et al.*, 2012; Fais *et al.*, 2015; Guerra *et al.*, 2017; Isojunno and Miller, 2018). These studies have focused on echolocation signals corresponding to dive behavior over short time windows of hours instead of days, and with small sample sizes. While acoustic and behavioral archival tags can help elucidate small-scale foraging tactics of individuals, inferring population-level movements over longer periods of time and over broader regions is difficult with these short-duration data sets that require collection of the tags to access data. Few studies have specifically assessed regional-level movements and habitat use by males foraging at high latitudes, coupled with diving and foraging behavior.

Individual movement of sperm whales is primarily centered around energetic requirements needed to grow, survive, and reproduce. Tracking individual movements and characterizing behavior can be used to better understand biological processes that influence food availability and occurrence of animals in general (Gurarie *et al.*, 2016; Hays *et al.*, 2016). In turn, this information can be used by managers to designate critical habitat, conservation zones and stock boundaries for highly migratory species, such as sperm whales (Schick *et al.*, 2009; Rosenbaum *et al.*, 2014; Lesage *et al.*, 2017). In the marine environment prey resources are often patchy due to complex interactions among environmental variables (e.g. sea surface temperature, tidal currents, light availability, bathymetry, etc.). Identifying the key environmental drivers influencing predator movement and habitat use can be used to predict changes in distribution due to a changing environment (Jay *et al.*, 2012; Joy *et al.*, 2015). However, observing these behaviors can be difficult for some species such as sperm whales, because they are often distributed far from shore, undergo deep dives that can last more than 30 minutes, and are often

hard to locate and track. Furthermore, surface observations rarely allow for visual confirmation of foraging habits, social interactions, or other ecological markers. Satellite telemetry through transmitting tags can be used to track more broad-scale movements, capture migratory routes, and elucidate foraging hotspots.

Argos satellite-linked transmitting tags can be used to gain valuable insight into broad movement patterns, how animals interact with their environment, and specific environmental characteristics that influence their behavior and occurrence (Arthur *et al.*, 2015; Lesage *et al.*, 2017; Riekkola *et al.*, 2019). Satellite tagging can provide researchers with detailed movement patterns to determine spatio-temporal foraging patterns and infer the quality of the environment that was visited by the tagged animals (Bestley *et al.*, 2013; Guinet *et al.*, 2014; Thorne *et al.*, 2017). Over the last few decades the application of bio-logging methods through satellite tagging has been used increasingly to better understand how free-ranging marine mammals use their habitat and interact with their environment (Jonsen *et al.*, 2013; Silva *et al.*, 2014; Hays *et al.*, 2016). SEASWAP has used satellite tags to gain information on how sperm whales use their GOA foraging ground habitats. Straley et al. (2014) assessed 11 satellite tag records deployed in the summers of 2007 and 2009 during SEASWAP research activities in the eastern GOA. Sperm whale movements were found to be strongly associated with the continental slope, with horizontal movement rates increasing when whales moved south out of Alaskan waters (Straley *et al.*, 2014). Nine of the 11 tagged whales remained in GOA waters while tags transmitted, while two of the tagged whales departed the GOA and moved south along the continental slope to Mexican waters before tags stopped transmitting. This preliminary analysis showed that movements of males in the GOA foraging grounds followed no obvious pattern and was variable among individuals (Straley *et al.*, 2014). However, additional tag data has become available since this study, and additional analyses to identify behavioral processes behind the observed movement are now possible to increase our understanding and interpretation of sperm whale movement in this region.

Interpreting movement data is complicated by animals moving in multidimensional space and autocorrelation among successive observations in time and space. Specifically, marine mammals are underwater for most of their lives and only come to the surface to breathe. Tags only transmit

when the animal is at the surface and the tag is exposed to air; therefore, transmissions occur at irregular intervals due to the diving behavior of the animal. Tag placement on the animal, behavioral and physical heterogeneity of individual animals, and occurrence of a satellite passing overhead while the animal is at the surface contribute to high variability in position estimates and associated error. For example, a tag placed low on the side of a whale might not fully come out of the water during a majority of the time the whale is at the surface, resulting in fewer messages being transmitted to any Argos payload satellites that are in view. A common way to deal with data gaps and variable error among position estimates is to interpolate positions at a fixed interval using modeling techniques such as state space models (SSM) (Jonsen *et al.*, 2005).

Recent advances in state-space modeling has allowed ecologists to interpolate location data, infer fine-scale movements, and estimate behavioral states from irregular, satellite-derived location data (Aarts *et al.*, 2008; Jonsen *et al.*, 2013). SSMs are a stochastic model-based approach to working with tag data that is designed to address measurement error, and separate the observation error inherent in the Argos system from the often random processes determining animal movement (Tanizaki, 2001; Jonsen *et al.*, 2003, 2005, 2007; Aarts *et al.*, 2008). They are a natural framework to apply to animal movement in that they estimate the state of an unobserved process (tagged animal movement and space use) from an observed data set (surface intervals that produce estimated positions from Argos data). SSMs rely on the notion of an animal's "state", or behavior, which can be categorized into classes such as "moving/transiting" and Area-Restricted Search (ARS). ARS behavior is often referred to as a "foraging" or "resident" state, and we will refer to it in this study as "foraging". These models assume that predicting future states is based on the current state, and model the randomness of an animal's movements concurrently with accounting for the randomness of the data collection. SSMs infer position estimates from observed satellite tag positions, while accounting for both errors in tag data and the stochastic movement process of animals.

In the current study, we increase the sample size of SEASWAP's satellite tag data set and expand the analysis to deal with the error associated with position estimates through state space modeling. We interpolate uncertain positions obtained from satellite tags to estimate positions at regularly spaced time intervals and estimate underlying behavioral states of sperm whales in this

region. In addition, we add dive behavior data from a subset of satellite tags equipped with depth sensors to quantify correlations between environmental drivers and diving behavior. Our goal was to better understand depredating male sperm whales' use of space in the GOA using satellite telemetry. Specific objectives were to: 1) describe general patterns in sperm whale movements and diving behavior; 2) characterize foraging and transiting behavior to identify foraging hotspots of sperm whales in the GOA; and 3) identify environmental predictors of sperm whale diving behavior during their presence in the GOA.

3.3 Methods

3.3.1 Field deployments

Satellite tags were deployed along the eastern GOA slope and in Chatham Strait between Cape Ommaney (56.15°N, 134.67°W), and Cross Sound (58.08°N, 136°W) between 2007 and 2016, roughly corresponding to the SEASWAP study area of the eastern GOA (Figure 3.1). Most tag deployment effort occurred in June and July, though deployments ranged from May 3 to September 17 (Table 3.1). All tags were deployed from small vessels at a distance of 3-15 m from the whale using two methods: between 2007 and 2009 tags were deployed using a crossbow; from 2010 to 2016 tags were deployed using a pneumatic rifle (DAN-INJECT JM SP 25, DanWild LLC). During tagging operations, we attempted to take photographs of dorsal fins and flukes (Whitehead and Gordon, 1986), to identify the individual whale in the SEASWAP catalog and ensure the same whales were not repeatedly approached and tagged in a single year. All tagged whales were determined to be male either from genetic samples collected from the individual, or based on size as determined by the tagging team. Tag IDs were given the prefix "SWsat" for "sperm whale satellite tag", followed by a numeric indicator assigned in chronological order based on date/time of tag deployment, while identification of individual whales to the SEASWAP photographic-identification (photo-ID) catalog was listed as the "Whale ID".

3.3.2 Tag programming – location data

A variety of tag types were used throughout the study, all configured as Low Impact Minimally Percutaneous External-Electronics Transmitters (LIMPET) (Andrews *et al.*, 2008) (Wildlife

Computers, Redmond, WA) (Table 3.1). All tags transmitted location information, while some tags also contained a pressure sensor, which recorded and transmitted dive information in addition to locations (Table 3.1). Tag size varied, with a maximum dimension of 5.5 x 5.0 x 2.7 cm and maximum weight of 57 g; two barbed titanium darts penetrated the skin and outer blubber layer up to 6.5 cm (Andrews *et al.*, 2008).

Tags transmitted to polar-orbiting satellites via the Argos satellite system. Tag programming parameters varied among years due to different project goals and objectives, and because tags evolved and improved over the years. In general, between 2007 and 2015 (SWsat1-SWsat28), tags transmitted every day for 19, 20, 45 or 50 days, before switching to a duty cycle to transmit every 2, 3, or 4 days for 10-40 days. Transmission repetition intervals varied between 10 sec and 30 sec. Thereafter, tags continued to transmit on one day out of every 5, 6, 8, or 10 days for the remainder of the tag attachment duration. In 2016 (SWsat29-SWsat33), the location-only tags (SWsat29, 31, and 32) were programmed to transmit every day for the entire tag life, but the hours the tag attempted to transmit were reduced from 22 hours per day for the first 50 days, to 7 hours per day for the next 15 days, to 3 hours per day for the following 15 days, and then to 2 hours per day for the remainder of the tag's attachment.

3.3.3 Tag programming – dive depth data

A subset of tags deployed between 2010 and 2016 also contained a pressure sensor to record and transmit depth information during dives (Mk10-A and SPLASH tags). These tags were programmed to collect and transmit dive data according to a duty cycle that varied with the year of deployment and SEASWAP project objectives (see Appendix A3.1). In 2010 and 2013 tags were programmed to transmit summaries of position and depth data for the first 20 days, before switching to a duty cycle, during which they transmitted data only from the dates of transmission. Duty cycles consisted of tags transmitting every 2, 3, or 4 days for 10, 24, or 30 days, and then every 8, 10, or 16 days for the remainder of the tag deployment. In 2014 tags were programmed to transmit daily for 19 days, and then switched to a duty cycle of every 3 days for 12 days, every 6 days for 30 days, and then every 12 days for the remainder of the tag deployment. The two tags in 2016 that had dive data (SWsat30 and SWsat33) were programmed to transmit daily. Tags summarized the dive data in two ways, sending messages summarizing

daily histograms of maximum dive depths, providing an accurate count of how many dives were performed each day, and as Behavior Log data that summarized the duration, maximum depth reached and shape for individual dives. Maximum dive depth was defined as the maximum depth reached in an individual dive. Start and end times of dives were determined using a wet/dry sensor, and dive information was collected on dives that qualified as "dives" according to a qualifying threshold of at least 30 m depth and 30 sec in length. The dive depth threshold was chosen to separate surface intervals and shallow silent dives from foraging dives, with the 30 m threshold equating to approximately two body lengths of an adult male sperm whale. Once a whale crossed the dive qualifying threshold for both depth and duration, the record was classified as a dive and dive characteristics were recorded and transmitted when the tag was at the surface. Dive characteristics were summarized by the tag as: start and end time of the dive, maximum depth of the dive, duration of the dive, and dive shape. Dive shape was assigned to each dive based on depths collected from a pressure sensor on the tag, sampled at 1-second intervals and classified as either V-shaped, U-shaped, or square-shaped (Appendix A3.1).

3.3.4 Satellite location filtering

All position estimates from satellite tags were derived by Argos using the Doppler effect to calculate a position. Positions were then filtered to assess each position estimate and remove improbable positions from the data set using the Douglas Argos-Filter, v. 7.06 (Andrews *et al.*, 2008; Douglas *et al.*, 2012). Prior to 2011, a non-linear Least-Squares algorithm was provided by Service Argos to calculate raw position estimates. In 2012, Argos began offering a new processing algorithm, the more robust Kalman smoothing method, which improved location accuracy, especially when a limited number of messages were received (Lopez and Malardé, 2011; Lopez *et al.*, 2014; Silva *et al.*, 2014). Tag data from 2009 and 2010 were re-processed by Argos using the Kalman smoothing method, thus only 2007 position estimates were calculated using the Least-Squares algorithm.

Most position estimates from Argos have an associated location quality class (LC) associated with them, which is linked to the estimated error from each position estimate and is referred to as the radius of error, forming an ellipse around the position. These LC's consist of 3, 2, 1, A, B, and Z, with 3 being the most precise estimate of position (i.e. the smallest error associated with

the position estimate) with a <250 m error radius, and B being the least accurate with unbounded accuracy estimation (CLS, 2016). LC codes of "Z" denote invalid positions.

3.3.5 Modelling locations using SSMs

We used a first difference correlated random walk model (DCRW) to fit sperm whale movement from the final tag data set. SSMs were fit using a hierarchical version of Bayesian switching state space modelling methods to predict locations of whales at regularly spaced time intervals and to estimate behavioral state (Jonsen *et al.*, 2005; Jonsen, 2016). We interpolated irregularly spaced location data using both a 1-hour and 3-hour time step, restricting models to the period of time when tags transmitted daily. Time steps were chosen considering both: 1) the relevant timescale over which behavioral changes are likely to be evident; and 2) the actual temporal resolution of the data. For the latter, the tag data produced an average of 12 position estimates per day, or an approximately two-hour time step. For the former, behavioral changes could occur at timescales of an hour or two as animals search for prey, but given that sperm whale dives can last nearly an hour, behavioral changes may not be evident at such short time steps. Additionally, at small timescales such as one or two hours, every step is small, with small turn angles, and a high degree of correlation to the previous step. Hence, every move becomes similar and there is little to differentiate two behavioral states and the data risks being described as only one state (foraging or transiting). Finally, small time steps also result in a higher degree of autocorrelation of positions when analyzing movement data. We experimented with time steps of 1-6 hours and used model diagnostics to ultimately select three-hour time steps to estimate behavioral states, bridging the gap between the temporal resolution of the tag data (12 position estimates every day equating to a 2-hour time step) and the temporal resolution that behavioral changes would likely be evident (every three or four hours). Hourly time steps to estimate positions from the models were also used in dive behavior analysis, due to their increased sample size and good agreement with position estimates from the three-hour time step. Estimating positions after tags switched to a less than daily duty cycle was not done due to increased uncertainty when irregular data becomes even more sparse.

Models were fit using the 'bsam' package in R (Jonsen *et al.*, 2005; Jonsen, 2016; R Core Team, 2019) using the software JAGS (Depaoli *et al.*, 2016) and the R package "rjags" (Plummer,

2016). Markov chain Monte Carlo (MCMC) chains were run in parallel for 50,000 simulations, first discarding 25,000 samples from the 'burn-in' phase. Samples were then thinned, retaining every 200th sample to reduce autocorrelation. Behavioral mode (b) was returned by the model as a value between 1 and 2, where values close to 1 ($b < 1.25$) were indicative of transiting behavior and values close to 2 ($b > 1.75$) were indicative of area-restricted-search (foraging behavior).

For the subset of tags that included dive statistics (n=14), hourly tag position estimates were matched to the closest dive if the time of the position estimate was within five minutes of the start or end of a dive, or during the dive. If position estimates were matched to a dive, they were associated with the maximum dive depth, dive duration, and dive shape information for further analysis.

3.3.6 Environmental data (explanatory variables for models)

Seafloor depths for each position estimate were obtained from the NOAA Alaska Fisheries Science Center's Central Gulf of Alaska raster, collected from lead-line and single-beam echosounder soundings from 225 National Ocean Service hydrographic surveys and compiled as a 100 m resolution ArcMap grid (https://www.fisheries.noaa.gov/alaska/ecosystems/alaska-bathymetry-sediments-and-smooth-sheets). For data points that were outside of the area covered by the NOAA bathymetry surface (approximately 30% of the data points), the Pacific GEBCO_19 gridded surface was used, which contains depths in a bathymetric raster surface in ArcGIS (GEBCO Compilation Group 2019; GEBCO 2019 Grid; doi:10.5285/836f016a-33be-6ddc-e053-6c86abc0788e) with a grid resolution of 15-arc seconds, or approximately 500 m.

Habitat was categorized into one of three categories: continental shelf, continental slope, and deep ocean basin. Continental shelf habitat was defined as nearshore depths between 0-300 m, continental slope was defined as the area where the continental slope drops off to the deep ocean basin with depths between 300 – 2000 m, and the deep ocean basin was defined as having depths greater than 2000 m (Weingartner *et al.*, 2009).

Slope of the bathymetry was calculated over a 1 nautical mile gridded area in the eastern GOA that roughly corresponded to the NOAA Fisheries Eastern GOA statistical area for sablefish

management, from 53°N Latitude to 60°N Latitude (https://www.fisheries.noaa.gov/alaska/sustainable-fisheries/pacific-halibut-and-sablefish-individual-fishing-quota-ifq-program). Slope calculations were conducted in GIS using the slope tool in the Spatial Analyst Tools toolbox, with output specified in degrees, using a z-factor conversion of 0.00001625 for latitudes around 56°N. Interpolated positions were then matched to grid cells and the slope was extracted from that cell.

We calculated the proportion of the water column used in dives by comparing maximum dive depths to bathymetry at those position estimates that had associated dive behavior information. We calculated the "distance to the seafloor" as the distance (in meters) between the maximum depth of each dive, and the seafloor depth at that position. Because dive characteristics were matched to within 5 minutes of an hourly position estimate, and because of uncertainties in both position estimates and bathymetry data, some dive depths were deeper than the seafloor depth estimated for that position. This was exacerbated by the fact that the area the sperm whales were inhabiting is characterized by steep continental slope gradients. Nevertheless, our sample size for modeled position estimates matched with dive information was large enough (n=6,345) to assess general patterns in the proportion of the water column whales used while diving.

Catch-per-unit-effort (CPUE) data for estimating local sablefish abundance was obtained from fishery observers on commercial longline fishing vessels participating in the sablefish fishery in the Southeast (SE) area of the GOA within the broader Eastern GOA statistical area, collected and compiled by the NOAA Fisheries Ted Stevens Marine Research Institute in Juneau, AK. Data represented catches from 1995 through 2017. A geospatial model of CPUE was developed in a generalized additive modeling (GAM) framework, which was used to predict CPUE over the interpolated sperm whale position estimates from SSMs within our study area in the eastern GOA (see Appendix A3.2). We assumed that spatial patterns in CPUE were consistent over the timeframe given in the data set, reflecting a long-term average CPUE at each position (Appendix A3.2).

Lunar phase was calculated as a continuous variable of lunar illumination from the 'lunar' package in R for each interpolated hourly tag position. For lunar illumination, 0 represents a new

moon, 0.5 represents quarter moon, 1 represents a full moon (100% illumination), crescent moons are between 0 and 0.5, and gibbous moons are between 0.5 and 1. Diel cycle was calculated for each hourly tag position as well, using the R package 'maptools', as day, night, dawn, or dusk. Nautical dawn/dusk were defined as starting or ending, respectively, when the angle of the sun was 12 degrees below the horizon.

3.3.7 Objectives

Objective 1: Describe general patterns in sperm whale movement and diving behavior

Broad patterns of horizontal movement and summary statistics on sperm whale tags were described for each tagged whale. Average movement speeds, the number of days tags transmitted data, and the general direction of movement were summarized, as well as any unique movements or tag tracks. Whale tracks were analyzed with respect to their movement in and out of the GOA, which we define as the region northward of 52° N Latitude, which coincides roughly with Queen Charlotte Sound and the southern end of Haida Gwaii, British Columbia (Brower Jr. *et al.*, 1988). In addition, dive behavior was summarized using the maximum depth, maximum duration, and an estimate of dive shape for each dive.

Minimum horizontal rates of travel (km/hr) and minimum daily distance (km) traveled were estimated as in Straley et al. (2014) to quantify daily movements. Briefly, straight-line distances between the best position estimate (based on Argos LC) for each day were first calculated, and then divided by the time between those estimates to get a minimum daily movement rate (km/hr). While this method does not account for all of the movement by the tagged animal (e.g. back-and-forth or circuitous foraging movement throughout a day), nor vertical movement during dives, it provides an estimate of the minimum rate of movement or minimum swimming speed required to travel between the most accurate daily positions. The minimum rate of horizontal movement was multiplied by 24 hr, to provide a minimum estimate of the distance traveled that day.

Objective 2: Characterize foraging and transiting behavior to identify foraging hotspots of sperm whales in the GOA

Foraging and transiting behavioral states were quantified from SSMs and filtered to only include data points where behavioral states (b) had a high degree of certainty, $1<b<1.25$ & $1.75<b<2$

(Jonsen *et al.*, 2013; Jonsen, 2016). Foraging and transiting behavioral states for each whale position were examined visually to see if we could identify foraging hotspots of sperm whales in the GOA. The proportion of transiting versus foraging states produced by the model was calculated, and t-tests were used to examine whether or not the proportion of position estimates in the foraging state within the GOA was different than outside the GOA (i.e. whether or not whales were spending more time foraging within the GOA than outside the GOA). We also tested differences in dive shape within and outside the GOA, as well as for foraging versus transiting behavioral states. Square dives provide the greatest bottom time, where we presume most foraging is happening. Therefore, we hypothesized that dives performed when the whale was in a "foraging" state were more likely to be square than when in the "transiting" state. Given we presume sperm whales spend time in the GOA primarily to feed, we hypothesized that square dives would be more prevalent in the GOA than outside the GOA.

Objective 3: Identify environmental drivers of diving behavior

To explore environmental drivers of diving behavior we restricted our analysis to the eastern GOA (Figure 3.1) to exclude areas to the south that may serve different purposes for sperm whales (i.e. migration). Moreover, this was the area for which we had available CPUE data for sablefish to use as a covariate in the model. Thus we clipped the SSM output to include only positions (interpolated from SSMs) within the GOA, removing 26% of the full data set. We further filtered the data to include only tag data for positions that had associated dive information, further reducing the data set by 40% and resulting in 5,090 data points used in the final analysis for this objective.

For the subset of tags that included diving data, we assessed the influence of environmental covariates on sperm whale diving behavior using generalized additive models (GAMs) fit via maximum likelihood estimation and allowing behavior to vary between individuals (Wood, 2017). For this analysis we used response variables of maximum dive depth and dive duration. Response variables were modeled as a function of seafloor depth (z), seafloor slope, lunar cycle (lunar), diel cycle (diel), day of the year (DOY), and an index of local sablefish abundance estimated from the fishery (Catch-per-unit-effort, CPUE) as follows:

$$y_{i,s,t} = \alpha + a_i + f_1(z_s) + f_2(slope_s) + f_3(lunar_t) + f_4(diel_t) + f_5(doy_t) + f_6(CPUE_s) + f_7(SST_{s,t}) + f_8(lat_s, lon_s) + \varepsilon_{i,s,t}$$

where $y_{i,s,t}$ is the response (maximum dive depth or dive duration) for tag i at position s and time t, α is an overall intercept, a_i is a random intercept for tag i to allow for individual variation in dive depth and duration, z_s is seafloor depth at position s, the $f_{1\text{-}7}$ are smooth functions with up to 3 d.f., f_8 is a bivariate smoother to account for any remaining spatial variation , and ε is the residual variation. The random effects a_i and residuals $\varepsilon_{i,s,t}$ are assumed to be normally distributed with mean zero and variance σ_a^2 and σ_ε^2, respectively.

A histogram of maximum dive depths across all tagged whales showed multiple modes, with a small mode of shallow dives between 30 m (our dive depth qualifying threshold) and approximately 125 m, a main mode between 125 and 800 m, and a broad mode of relatively few deep dives ranging from 800 m to the maximum dive depth. All of the deeper dives were produced by a subset of whales, while the maximum dive depths of most whales were less than 800 m. Initial efforts to model the full depth distribution were not successful in explaining the depth of shallow or deep dives, resulting in large, influential negative and positive residuals, respectively. To address distributional assumptions and individual variability, we visually selected cutoff depths of 125 m and 800 m based on gaps in the depth distribution of the full dive data set and modeled the depths of 'typical' dives (called intermediate dives) between 125 m and 800 m. Models were fit with a GAM using a Gaussian distribution with an identity link. In addition, we separately modeled the probability of shallow dives (< 125 m) and the probability of deep dives (> 800 m) as a function of the same covariates, using two binomial models with a logit link. All models for dive depth and duration were fit using the 'mgcv' package in R v 3.6.2 (R Core Team, 2019; Wood, 2017) and were limited to the general eastern GOA study area corresponding to the sablefish management area as described above. A stepwise model selection procedure using the Akaike Information Criterion (AIC) was used to choose the most parsimonious model (Burnham and Anderson, 2002). Residuals from the global model and from the best-fitting model were visually examined and statistically tested for spatial autocorrelation within a given year by fitting exponential and spherical variograms to the residuals. We found no

evidence of significant spatial autocorrelation in the residuals from either model, thus errors were assumed to be independent after accounting for the effect of the covariates.

3.4 Results

3.4.1 Tag deployment summary

A total of 33 satellite tags were deployed on 28 individual male sperm whales in the eastern GOA between 2007 and 2016 (Table 3.1). Tags were primarily deployed during the summer months; the earliest tag deployment was May 3, and the latest deployment was September 17, with a majority of tags being deployed in June and July (n=20). Of all tags deployed, one (SWsat23, 2014) failed to transmit, likely due to insecure attachment; one tag (SWsat25, 2014) hit the whale very low on the body and, though it stayed attached for at least 27 days, no more than one message in a satellite overpass was ever received so no position estimates could be calculated. Two tags (SWsat1 and SWsat3, 2007) transmitted for nine and three days respectively, with each tag transmitting only five total position estimates. We deemed these tag records too short to use in the analysis. Thus, of the 33 tags deployed, there were 29 deployments on a minimum of 26 unique individuals (two whales were not identified) with usable tracks to assess movement of whales (Table 3.1). No whale was knowingly successfully tagged more than once in a single year. Sixteen of the tags recorded dive depth data, though we did not receive any dive data from two of them (SWsat23 and SWsat25), leaving 14 tags with dive depth data for analysis (Table 3.1). The remaining 15 tags used in analyses were location-only tags (Table 3.1).

The 29 usable tag records we had gave us the ability to track sperm whale movement over the attachment period for each tag (Figure 3.2). Tags stayed on sperm whales an average (±SD) of 43 (±42) days, ranging from 3 days (SWsat7) to 164 days (SWsat26) (Table 3.1). The number of usable position estimates per day averaged 12 (± 7). Two whales, GOA-047 and GOA-050, were tagged in two different years. GOA-047 was SWsat3 in 2007 and SWsat7 in 2009, however, the first tag (SWsat3, 2007) remained attached for only three days and recorded five positions, and thus was not used in this analysis (Table 3.1). GOA-050 was SWsat14 in 2010 and SWsat25 in 2014, but in 2014 the tag did not transmit positions successfully, likely due to the tag's location being too low on the animal to successfully transmit enough messages to satellites per surfacing

to obtain a position estimate (Table 3.1). A third whale, GOA-091, was tagged in four different years: 2010, 2014, 2015, and 2016 (Table 3.1, Appendix A3.3). Three of the four tag deployments for GOA-091 had dive depth data recorded (Table 3.1, Appendix A3.3).

3.4.2 Objective 1: General patterns in sperm whale movement and diving behavior

Regional Movement

Of the 29 whale tracks analyzed, nearly 1/3 (n=10) generally moved northwest, out of the study area and towards the Central GOA (Figure 3.2). The remaining whales either stayed in the study area (n=5) or moved south (n=14) while the tag was on the whale. Nine of the tagged whales moved south past the southern tip of Haida Gwaii (~52°N Latitude) into Queen Charlotte Sound waters before the tags stopped transmitting. Of the whales that moved south and out of the GOA, none turned around and moved northward again at any point while tags were transmitting. However, three tagged animals turned around and spent time off Haida Gwaii between 52°N and 54°N Latitude. Tagged whales primarily left the GOA heading south during summer and fall months, with two whales heading south in June, one in July, one in August, one in September, three in October, and one in January. Five tags stayed on whales that moved south of Washington state. These animals all moved to points offshore of California or Mexico before the tags stopped transmitting (Supplemental Data S3.1).

Horizontal movement

The minimum daily rate of horizontal movement across all tagged whales had a median of 1.7 km/hr (38.6 km/day) but was highly variable, ranging from 0.04-7.79 km/hr (mean = 2.2 ±1.8 km/hr). For the tagged whale positions that were within the GOA (north of 52°N Latitude), the median minimum daily rate of horizontal movement was 1.4 km/hr (33 km/day), similar to the overall median. However, when considering only tagged whales that moved out of the GOA, the median minimum daily rate of horizontal movement increased to 5.5 km/hr (123 km/day) after whales left the GOA, indicating whales had a tendency to speed up or move in a more linear fashion after leaving the GOA (Figure 3.3).

Seafloor depth under tagged whale positions averaged 768 m (± 716 m), with 75% of all positions being classified as over the continental slope, 18% over the continental shelf, and 7%

over the deep ocean basin. Average seafloor depth at interpolated sperm whale position estimates within the GOA (651 m) was shallower than outside the GOA (1806 m) (Figure 3.3).

Use of inside waters

In 2010 two whales (SWsat13 & SWsat15) that were tagged in the northern part of the study area moved south along the continental shelf edge after tags were attached, and then turned into inside waters of Chatham Strait in Southeast Alaska. This occurred in late August, while the state-managed sablefish fishery was taking place in Chatham Strait (Figure 3.4a). In 2014 SEASWAP focused some tagging efforts in Chatham Strait to specifically target animals that were using inside waters while the fishery was taking place. Two whales were tagged in Chatham Strait in 2014, one in 2015, and one in 2016. Over all the years SEASWAP has documented sperm whales in Chatham Strait (2010, 2014, 2015, and 2016), only three different individuals have been identified using photo-identification. These individuals are GOA-023 (SWsat13), GOA-091 (SWsat15, SWsat27, SWsat28, and SWsat33), and GOA-086 (SWsat26). In 2015, one of these tagged whales, GOA-091 (SWsat28) circumnavigated Baranof and Chichagof Islands (Figure 3.4b). Another of these whales, GOA-086 (SWsat26), traveled northward into Lynn Canal, moving outside of the commercial sablefish fishery area (Figure 3.4c).

Dive behavior

Fourteen tags provided diving information, resulting in 7,573 total dives for this analysis. On days when a tag collected and transmitted dive data, we received detailed Behavior Log information for an average of 81 ± 22 % of all dives made each day. Information about some dives was missing because not all of the messages produced by the tag are received by an Argos satellite, usually because satellites are overhead for such a small proportion of time, but also because of the limited opportunities for transmission when the whales spend so much time submerged. Dive durations and maximum dive depths appeared to be similar among different individuals (Figure 3.5), with the mean maximum dive depth ranging from 350 m to 507 m across the fourteen tag records (overall average = 396 m ± 116 m) and the mean dive duration ranging from 29min to 38min (overall average = 32 min ± 9 min) (Table 3.2). The maximum recorded dive depth was an 1848 m dive by SWsat13 in October of 2010. The longest dive recorded was a 112-minute dive in May 2013 (SWsat20). GOA-091, the individual tagged four

times, had dive data recorded for three of its tags (SWsat15, SWsat27, and SWsat33). The mean dive depths of these three deployments varied considerably, at 507 m, 406 m, and 369 m respectively (Table 3.2, Appendix A3.3).

A majority of dives for all tag records combined were square-shaped (71%), followed by U-shaped dives (23%), and V-shaped dives (6%) (Table 3.3). This pattern was fairly consistent across all individual tags that recorded diving information, though there was some variability in the proportion of each dive shape an animal exhibited (Appendix A3.5). Maximum dive depth varied significantly with respect to dive shape (ANOVA; F=89.38, p<0.001), where U-shaped dives were the deepest. V-shaped dives, while of similar average duration, were significantly shallower on average than square or U-shaped dives. Seafloor depth varied significantly with respect to dive shape as well (ANOVA; F=5.46, p=0.004) with V-shaped dives in the deepest water and square-shaped dives performed over the shallowest depths (Table 3.3).

3.4.3 Objective 2: Characterize foraging and transiting behavior to identify foraging hotspots of sperm whales in the GOA

Raw position data for the period of time the tags transmitted daily (8,729 positions) were analyzed with state-space models to interpolate an estimated position every three hours, resulting in a total of 5,817 position estimates. Diagnostics indicated models converged well; within-chain sample autocorrelation was low and Brooks-Gelman-Rubin scale reduction factors were <1.1 for all tags.

To characterize foraging and transiting behavior the full SSM output data was further filtered to include only data points where behavioral states had a high degree of certainty, 1<b<1.25 and 1.75<b<2 (Jonsen *et al.*, 2013; Jonsen, 2016). This resulted in a total of 4,505 positions with an estimated behavioral state. Overall, behavior was primarily classified as foraging (74% of positions) versus transiting (26% of positions). Within the GOA, models predicted primarily foraging behavior, with 82% of interpolated positions classified as foraging versus 18% transiting. Outside the GOA, whale behavior was presumed to be primarily transiting, with 74% of interpolated positions being classified as transiting versus 26% foraging.

Of the individual dives recorded in the GOA, 75% were square-shaped, 19% were U-shaped, and 6% were V-shaped. Meanwhile, of the individual dives recorded outside of the GOA, 62% were square-shaped, 35% were U-shaped, and 3% were V-shaped. A Pearson's Chi-squared test revealed there were not significant differences in the dive shape within or outside the GOA (X^2=4.76, df=2, p=0.93). Square-shaped dives made up 78% of transiting dives and 74% of ARS (foraging) dives. A Pearson's Chi-squared test revealed no significant differences in the dive shape for ARS versus transiting dives (X^2=5.82, df=2, p=0.06).

Habitat

Seafloor depth at modeled hourly tag positions averaged 830 m (± 789 m; median=570 m), with 18% of all positions being classified as over the continental shelf, 73% over the continental slope, and 9% over the deep ocean basin. The slope of the bathymetry in 1 nm grid cells under tag positions averaged 5.7 degrees (± 4.9 degrees).

Proportion of water column used in diving

The proportion of the water column all individual whales used in diving ranged from 0.02 (2%) of the water column to 9.7 (970%) of the water column (proportions from 0-1 are from whales whose maximum dive depth was *less than* the seafloor depth, and proportions 1-9.7 are from whales whose maximum dive depth was *greater than* the seafloor depth). The median proportion of the water column covered by whales during individual dives was 0.84 (mean = 0.95 ± 0.65). To explore potential relationships between the proportion of the water column used by whales and light availability we performed an analysis of variance which indicated that the proportion of the water column whales used in a given dive was not significantly related to light availability (dawn, day, dusk, night) (F=1.16, p=0.33).

3.4.4 Objective 3: Identify environmental drivers of diving behavior

Binomial models for shallow dives (dives less than 125 m) were statistically significant for some explanatory predictors but most of the deviance was due to variations among individuals, followed by variation in sablefish CPUE (probability of shallow dives decreased with increasing CPUE). Similarly, binomial models for deep dives were statistically significant for some predictors but effects were poorly estimated due to a high number of probabilities close to zero

as a result of the small sample size of deep dives. Additionally, only a few tagged animals exhibited deep dives (SWsat13, SWsat14, SWsat15, and SWsat18). Thus, we focused our analysis on 'typical' dives, between 125 m and 800 m, which we refer to from this point forward simply as 'intermediate dives'. Day of the year (DOY) was confounded with individual variability among whales, due to records being obtained for different parts of the year. Therefore, the effect of DOY could not be resolved and it was removed from the model.

The best model for intermediate dive depths was the full model, including light level, lunar cycle, seafloor depth, slope, CPUE, and individual tag ID (Table 3.4). The model explained 33% of the deviance, with most of the deviance explained by seafloor depth, followed by light level, CPUE, lunar cycle, individual whale variability, and slope (Table 3.4). Seafloor depth was most strongly related to maximum dive depth, explaining 22 to 24% of the deviance in the model, followed by CPUE (6-10% of the deviance) and individual whale ID (1-6% of deviance) (Table 3.4). Dives tended to be slightly deeper during the day and at night, as well as during quarter moons, when tidal currents are weakest (Figure 3.6). Dive depth increased with seafloor depth up to about 800 m before decreasing over deeper water, although variability was high. Average dive depth decreased slightly as the slope of the seafloor increased. CPUE and dive depth were inversely related, with dive depth decreasing in areas of higher sablefish CPUE (Figure 3.6).

The best model for dive duration included all predictors except slope (Table 3.4) and explained 18% of the deviance. Most of the variability was attributed to differences in mean dive duration among individual whales ('DeployID'), which accounted for 10% of the overall deviance. Seafloor depth explained 3-5% of the deviance and sablefish CPUE explained 2-4% (Table 3.4). Dive duration varied little among light levels but showed a pattern of slightly longer dives during crescent to quarter moons (Figure 3.7). Dive duration increased as seafloor depth increased, but the estimated relationship leveled above approximately 1,000 m. Durations decreased as CPUE increased, with the shortest dives at a CPUE of approximately 0.3 kg per hook, and then dive durations increased again as CPUE kept increasing. Individual variability was significant, where a few individuals had longer dives on average (SWsat16 and SWsat27 (Figure 3.7)).

3.5 Discussion

We found that sperm whales in the GOA migrated over long distances, mostly stayed over the continental slope and did not migrate across the deep ocean basin (at least during our study period). They spent most of their time in the GOA foraging, and most of their time transiting when they left the GOA heading south. Whales in the GOA spent a large proportion of their time diving to the bathypelagic zone, though they also dove to the mesopelagic zone. Whales dove deeper and longer during the daytime and during quarter moon lunar cycles. Dives became shallower in areas of increasing sablefish CPUE. Maximum dive depth increased as seafloor depth increased, up to approximately 800 m seafloor depth, at which point dives became shallower again. Individual variability in dive depth and duration was fairly high.

3.5.1 General movement patterns

The timing of sperm whale migrations, specifically movement of males, is largely unknown (Whitehead, 2003). Tagged whales in our study made broad movements along the continental slope, with little movement over the deep ocean basin. One-third of whales tagged in the eastern GOA moved north towards the central GOA, while just over half of the whales moved south after tags were deployed and before they stopped transmitting. Nine tagged whales left the GOA heading south and did not turn around after moving south of 50°N Latitude while the tags were transmitting. Interestingly, these whales left Alaska during a variety of months and seasons (summer, fall, and winter). Two left in June (both 2009), one in July (2013), one in August (2010), one in September (2010), three in October (2009 & 2010), and one in late January (2015). Thus even within the same year (2009 and 2010) tagged animals that moved out of Alaska did so in different months, sometimes as far apart as late June to late October (2009). This data set consists of whales tagged from May to September, which biases our information on timing of migrations to the summer, fall, and winter seasons due to an average tag deployment duration of 45 days. However, even with the limited seasonal tag deployments, our data suggests that male sperm whales in the GOA do not exhibit predictable seasonal migrations.

Tagged whales that left Alaskan waters headed south toward warmer equatorial waters off Mexico, and some went into the Sea of Cortez where breeding female and juvenile groups are known to congregate and mature males have been seen (Jaquet and Gendron, 2002, 2003, 2009;

Ruiz-Cooley *et al.*, 2004; Davis *et al.*, 2007). Behavioral state of tagged whales switched from primarily foraging in the GOA waters to primarily transiting when they left GOA waters heading south. Whales also sped up after leaving the GOA, further indicating this change in behavior, consistent with findings from Straley et al. (2014). These findings suggest the GOA is an important foraging area for these individuals, in that they did not appear to spend time in any other regions during their migrations and while tags were transmitting.

The use of inside waters of Chatham Strait by multiple tagged whales, representing three individuals, has not been previously documented in this region. Chatham Strait is a deep fjord with depths exceeding 700 m, which are well within the depth range sperm whales inhabit in their offshore habitat. The State of Alaska manages a limited entry sablefish fishery in Chatham that is open from mid-August to mid-November each year. The individual that circumnavigated Baranof and Chichagof Islands (SWsat28) moved through shallower waters in Icy Strait that are not typical sperm whale habitat, where sperm whale presence had not been confirmed previously (Figure 3.4b). The individual that moved north into Lynn Canal (SWsat26) also ventured into shallower waters that were previously undocumented habitat for sperm whales (Figure 3.4c). The two whales (SWsat13 and SWsat15) tagged in early August 2010 offshore of the northern region of our study area in the eastern GOA moved south together and entered inside waters of southern Chatham Strait a few weeks after being tagged (Figure 3.4a). The state-managed sablefish fishery was open in Chatham Strait during that time, and it is possible that both whales followed a fishing vessel into this narrow, inshore, deep canyon habitat. These tracks represent a continued association of two specific male sperm whales over a period of 43 days and these same two individuals were again sighted together near a fishing vessel in Chatham Strait in 2014 and in 2015. Only one other individual was sighted with them in 2014. Though male sperm whales are thought not to form associations (Lettevall *et al.*, 2002; Mizroch and Rice, 2013), we believe that this may be a unique example of a long-term association between two individuals. Alternatively, it could be that these two individuals were simply exploiting the same opportunity and were coincidentally sighted together across multiple years. Genetic sampling of these individuals could reveal more information about their relatedness.

The maximum dive depth and dive duration of sperm whales in the GOA averaged 396 m and 32 min for all dives analyzed in this study. This is within the observed range of dive depths and durations documented for both males and females in the Gulf of California (Davis *et al.*, 2007; Gallo-Reynoso *et al.*, 2009; Irvine *et al.*, 2017), and on the shallow end of recorded dives from male sperm whales in other high latitude regions such as Norway (mean 492 ± 593 m) and Kaikoura, New Zealand (mean 924 m) (Teloni *et al.*, 2008; Guerra *et al.*, 2017) (Table 3.5). A single individual tagged four times across six years (with three years containing dive behavior information) had variable maximum dive depths each time it was tagged. GOA-091 was tagged in 2010, 2014, and 2016 with a satellite tag containing and pressure sensor, and the average maximum dive depth the animal dove to was 507 m, 406 m, and 369 m respectively. Further, this variability encompassed the observed variability across all whales, with GOA-091 exhibiting the shallowest average (± SD) maximum dive depths (396 m ± 160 m) of all tagged whales, and also the deepest maximum dive depths (507 m ± 223 m) of all tagged whales. This suggests that variability in maximum dive depth may not relate to individual preference but instead may be more related to other factors (e.g. the location where individuals are tagged, where they spend their time during tag deployment, etc.).

Dives consisted mostly of square-shaped dives across all tags (Table 3.3). Interestingly, V-shaped dives and U-shaped dives had the same average duration (28 min), but V-shaped dives were on average over 100 m shallower than U-shaped dives (Table 3.3). This indicates they are likely used for different purposes than square and U-shaped dives, with whales spending very little to no time in the bottom phase of V-shaped dives (<20% total dive time, Appendix A3.1). Because V-shaped dives had the same duration, but their maximum depth was much shallower, we contend that these dives likely serve a transiting or searching purpose, but likely do not indicate successful foraging. Irvine et al. (2017) found V-shaped dives to have a similar depth as our study (median maximum depth of 290 m) and found that the depth was highly correlated to the depth of the preceding foraging dive, indicating they were likely intended for searching. Square-shaped & U-shaped dives both likely represent foraging dives and given their similarities could be grouped. Other studies exploring dive shape have different categories and classifications of dive type, making it difficult to compare across studies (Amano and Yoshioka, 2003; Fais *et al.*, 2015; Irvine *et al.*, 2017). In the future, using dive shapes, durations, and

maximum depths in behavioral modeling, when available, could help determine behavioral states of this species.

Much of the dive behavior for male sperm whales in high latitudes has been studied off the coast of Norway and New Zealand, using short-duration archival tags attached to whales for a number of hours, rather than days, using suction cups. Madsen et al. (2002b) used acoustics as a research tool to suggest that eavesdropping on conspecifics likely plays an important role in locating food aggregations for males in high latitudes. Other studies have shown that foraging creaks (an acoustic signal thought to be associated with prey capture attempts) were associated with whales rolling onto one side, suggesting sperm whales do not use visual cues to hunt, but rely mainly on echolocation and may have a preferred approach angle for echo-guided prey capture (Miller *et al.*, 2004; Fais *et al.*, 2016). Four sperm whales tagged with acoustic suction-cupped tags off the coast of Norway primarily dove shallower than 400 m, and bottom reflected echoes from echolocation clicks showed that they did not dive to the seafloor, indicating they may have been targeting epipelagic prey; in contrast, the few dives that did go to the seafloor likely indicated bathypelagic feeding (Teloni *et al.*, 2008). Overall, these studies show that male sperm whales in high latitudes forage in both bathypelagic and mesopelagic zones, they often switch between the two during a single day, and preference for foraging zones in the water column is likely a reflection of varying productivity and prey availability (Teloni *et al.*, 2008; Fais *et al.*, 2015, 2016; Guerra *et al.*, 2017; Isojunno and Miller, 2018). Our results show whales dive to a variety of depths and occasionally switch between depths somewhat abruptly (Figure 3.8, Supplemental Data S3.2), consistent with these other studies. Our findings that whales do not always dive to the seafloor to target prey also matches acoustic data from SEASWAP. Using surface and bottom-reflected echoes from clicks recorded on autonomous hydrophones to calculate depth of clicking whales, SEASWAP studies found whales were not diving to the seafloor when naturally foraging (i.e. not depredating) (Thode *et al.*, 2006).

We found no relationship between time of day (dawn, day, dusk, night) and the proportion of the water column whales dove to, indicating they were not consistently diving deeper during the day and shallower at night to follow diel movements of prey. This may be due to the time of year these tags were transmitting (mostly summer), and the limited darkness at high latitudes during

summer, which may reduce diel movement of prey. Diet analysis from depredating sperm whales in the GOA has shown primary diet items to consist of sablefish, dogfish, skates, and rockfish (Wild et al. 2020). Sablefish, dogfish, and some skates are known to exhibit diel vertical migration (Carlson *et al.*, 2014; Peklova *et al.*, 2014; Sigler and Echave, 2019), but little is known about vertical movement of other top prey items, such as rockfish and other skates. Thus, when diving to a specific depth range becomes less successful for sperm whales, they may switch to a different depth range to try to find a more suitable prey patch. Previous studies using acoustic tags on male sperm whales in high latitudes of the North Atlantic have discovered that whales begin clicking later in deeper dives, suggesting they use knowledge from previous dives to decide how deep to dive on the following dives (Teloni *et al.*, 2008; Fais *et al.*, 2015). Further, our results suggest similarities between this study and other research showing male sperm whales in high latitudes switch between prey layers several times per day (Supplemental Data S3.2) (Teloni *et al.*, 2008; Fais *et al.*, 2015).

3.5.2 Dive behavior modeling

Maximum dive depth and dive duration were most strongly related to seafloor depth (22% and 5% of deviance explained, respectively), individual Tag ID (6% and 10% of deviance explained), and CPUE of the sablefish fishery (6% and 4% of deviance explained). Other significant predictors to both models included light levels and lunar cycle, though these variables explained less deviance (Table 3.4). Slope was not strongly associated with dive depth and was not included in the best model of dive duration, suggesting the steepness of the seafloor is of little to no importance in sperm whale diving behavior.

We found that maximum dive depth decreased as sablefish fishery CPUE increased, which was a surprising result (Figure 3.6). In our separate model of sablefish CPUE (a measure of sablefish abundance), we found sablefish CPUE increased with increasing seafloor depths before decreasing below ~1100 m (Appendix A3.2), so dive depth would be expected to increase with sablefish CPUE if whales were naturally foraging for sablefish. However, models suggested shorter and shallower dives with increased CPUE, which may indicate depredation behavior, where whales do not need to dive as deep or as long when feeding from longline gear. Areas of high sablefish CPUE may be associated with increased fishing activity and may therefore attract

depredating whales. Whales are attracted to the high CPUE areas by either the high sablefish abundance, or the easy targets presented by fishing boats.

Lunar cycle and time of day (dawn, day, dusk, night) were less influential but still significant predictors of maximum dive depth and duration. These variables tend to be important to diel movements of prey in the ocean in general, where the deep scattering layer (DSL) undergoes diel vertical migrations (DVM) and rises from deeper water to more mesopelagic and subsurface waters at night (Eyring *et al.*, 1948; Johnson, 1948). There is little consensus on diel behavior of diving sperm whales in the literature (Irvine *et al.*, 2017; Stanistreet *et al.*, 2018). Guerra et al. (2017) found some evidence of different dive depths targeted between day and night, measured using acoustic buzzes (foraging creaks). For this study, we found that sperm whale dives in the GOA were deeper and shorter during the daytime than at dawn, dusk, or night, indicating potential responses to movement of prey. The mesopelagic zone in high latitudes has been found to extend between 100-400m during the day, rising above 200 m at night, and also exhibits seasonal fluctuations (Cooney, 1989; O'Driscoll *et al.*, 2009; Klevjer *et al.*, 2016). In the GOA, there is evidence of diel vertical migrations for many sperm whale prey species, including sablefish, rockfish, and spiny dogfish (Hunsicker *et al.*, 2010; Carlson *et al.*, 2014; Tribuzio *et al.*, 2017; Sigler and Echave, 2019). For sablefish in particular however, reverse DVM is also shown in this region, with fish rising in the water column during the day and descending at night (Sigler and Echave, 2019). Our results likely reflect changes in sperm whale foraging on their primary groundfish and squid prey as they respond to changes in light levels. The fact that diel cycles were less influential to the model may simply be a result of the restricted summer sampling season when there is very little darkness, as well as the lack of large differences between day and night vertical excursions for some of their prey.

Lunar cycles can influence prey movement as well because full moon phases are associated with more light than new moons, affecting the degree of DVM. In addition, lunar cycles affect tidal cycles and oceanic currents. A recent study on the diving behavior of pilot whales off the Hawaiian islands revealed that during full moon phases, pilot whales performed dives that were deeper, longer, and farther from shore than during new moons, potentially reflecting vertical movement of their primarily squid prey (Owen *et al.*, 2019). For our study, dives did not appear

to reflect differences between full moon or new moon phases. Instead, dives were slightly deeper and longer during the crescent to quarter moon phase, when tidal currents are weakest, indicating sperm whales in this region may be responding more to currents, rather than light. This could be due to a variety of factors including differences between species, habitat, and/or differences in prey preferences. Heavy cloud cover and storm systems obscuring illumination from lunar cycles in the GOA could reduce the influence of lunar phase on sperm whale prey, and thus the diving behavior of sperm whales. Research on zooplankton has indicated that while it likely cannot explain all of the variability in responses to lunar cycles, cloud cover and changes in light can explain some variability in zooplankton responses to lunar cycles (Last *et al.,* 2016). Sperm whale prey differs from that of pilot whales in that they consume a larger proportion of groundfish than squid in our study area (Wild *et al.,* 2020), which may not respond to lunar illumination in the same manner as squid, though research on lunar cycles with respect to groundfish movement in this region is lacking.

3.5.3 Additional considerations

Assessing sperm whale use of the water column

Estimating how close to the seafloor whales were diving was difficult for several reasons that hindered our ability to confidently assess how sperm whales use the water column in this area. First, whales may move large distances while diving and their position at the surface before and after a dive does not necessarily reflect their position during the bottom phase of their dive. Additionally, error associated with the proportion of the water column used in dives result from four likely causes which were inherent in these data:

a. Errors associated with satellite tag position estimates
b. Modelling error inherent in interpolating tag positions using SSMs
c. Variability associated with matching dives that averaged 32 min in duration (plus 5 min buffers at the beginning and end) to a single estimated point position.
d. Errors in seafloor depth estimated from low resolution bathymetric mapping in regions of steep gradients, particularly over the continental slope.

Maximum dive depth exceeded the estimated seafloor depth for 37% of our data points. Other studies have assumed that whales were diving to the seafloor at those times and assigned a seafloor depth equal to dive depth at those positions (Irvine *et al.*, 2017). However, because all depths are uncertain, truncating seafloor depths that exceed the associated dive depths introduces a bias in the average ratio of dive depth to seafloor depth towards lower values. If errors are symmetrical (i.e. seafloor depth at the approximate location as a given sperm whale dive is just as likely to be overestimated as underestimated), we argue that the median ratio of dive depth to seafloor depth provides a better estimate of the use of the water column. Using this approach we found that sperm whales on average use 84% of the water column when diving.

Seasonal variability in dive behavior

Seasonal differences in dive duration and maximum dive depth were confounded with variability in depth preference among individuals that were tagged in different seasons. Some whales dove deeper on average than other whales, and these whales were tagged later in the season. It is unclear if the deeper dives reflected differences in dive behavior among individuals or seasonal differences in dive depth. The average tag transmission duration when tags were both transmitting daily and diving behavior was available for any given tag was too short to resolve seasonal differences in diving behavior in this analysis.

Behavioral state models

The behavioral state space models we used worked fairly well in making broad comparisons of foraging and transiting behavior within and outside the GOA but may not be ideal for identifying more fine-scale foraging hotspots of sperm whales. These models have worked well for estimating foraging and transiting behavior of other marine mammal such as pinniped species and some baleen whales (Jonsen *et al.*, 2013; Lesage *et al.*, 2017). Many pinnipeds, such as fur seals and sea lions, are considered central place foragers: animals that clearly transit from the haul-out site to a productive area where they spend time foraging, before transiting back to the haul-out site. Tagging studies from these species have successfully used SSMs to identify transiting behavior between prey patches, and to and from haul-out sites, as well as foraging behavior in prey patches (Jonsen *et al.*, 2013). These models have also been useful with baleen whales that seasonally migrate between breeding grounds and feeding grounds, and have

identified foraging stopover locations used along migration routes (Lesage *et al.*, 2017). These studies identify switching of behavioral states at low resolutions, assuming behavioral changes occur at time steps of six hours or more. For this study, we were interested in identifying foraging hotspots within the GOA region, where switches between transiting and foraging could be happening at much smaller time scales. In addition, sperm whale prey of groundfish and squid may not be found in dense aggregations such as is found with krill or herring for baleen whales. Thus, their switches between foraging and transiting may be more subtle, and happen at shorter time scales. Finally, these whales may also be more graze-as-you-go foragers, constantly foraging and searching for prey on dives, even while transiting or migrating. This may be evidenced by the fact that even when they left the GOA, tagged whales continued to perform deep dives. Animals focused solely on transiting may choose not to expend the energy to make deep dives.

The field of analyzing movement data is rapidly evolving, with new models and methods being tested and modified constantly. There are two new versions of SSMs that may be useful for this data set but came out too recently to be included in this analysis. The first is still limited to two behavioral modes (foraging versus transiting), but uses a mixed-effects modeling approach that uses fast estimation tools rather than Bayesian methods and a continuous-time correlated random walk (CTCRW) rather than discrete-time correlated random walk (DCRW), which allows models to run more quickly and more accurately estimate space use of animals (Jonsen *et al.*, 2019). The second also uses CTCRW, but allows for additional behavioral modes, such as "resting", "hauled-out", or "searching" behaviors to be estimated, with user input conditions for these behaviors (McClintock *et al.*, 2014; McClintock and Michelot, 2018). Additionally, these newer models allow users to input additional covariates to be used in behavioral state estimation, such as dive behavior data, haul-out data, and environmental covariates (McClintock and Michelot, 2018). These methods have primarily been used with pinniped tag data, and we were unable to successfully adapt them to our sperm whale tag data set within the timeframe of this dissertation. However, the data set from this study that includes dive behavior may be applied in newer models successfully in the future to more accurately predict foraging versus transiting behavior for these sperm whales. If sperm whales are truly graze-as-you-go animals, it is also possible that the models would still be limited in their abilities to capture behavioral states of

these animals and state estimation would remain fairly uncertain because whales would be foraging and searching for prey while transiting. Nevertheless, exploring the use of these newer models would be useful for this data set in the future.

Linking tag data to depredation

One of our interests in the tag data was to identify depredation behavior for tagged whales, especially those whose tags recorded dive information. Previous research from SEASWAP using short-duration acoustic tags has shown that shallow depredation dives are significantly shallower (mean 145 m) and shorter in duration (mean 14 min) than deep depredation (487 m and 28 min) or natural foraging dives (439 m and 33 min) (Mathias *et al.*, 2012). In this analysis, we hypothesized that we might find evidence of a subset of dives that matched shallow depredation diving characteristics. However, we were unable to identify specific diving behaviors that clearly indicated depredation from the tag data alone. In fact, the multi-modal distribution of dive depths showed fewer dives between 80 and 160 m depth, where we expected to find a peak to indicate shallow depredation diving behavior, based on shallow depredation diving behavior findings from Mathias et al. (2012). Unless the animals were being observed visually during the entire tag deployment to confirm when they were depredating, there was no way to know what portions of tag data were definitely associated with depredation. Further, deep depredation dives, also characterized by Mathias et al. (2012), were only distinguished from natural foraging dives based on click and creak rates (acoustics) and proximity to fishing vessels. Thus, using this tag data alone to identify depredation behavior does not appear to be feasible at this time, but our findings do indicate sperm whales have an affinity for the continental slope habitat that overlaps with longline fishing habitat.

3.5.4 Conclusions

This study explored satellite tag data from a high latitude male sperm whale population, including behavioral state estimation and dive behavior analysis over longer time periods. The findings from this work increases our knowledge of sperm whale population dynamics in the North Pacific and has important management implications. Our results suggest that the GOA is an important foraging ground for these male sperm whales, and tagged whales spent a

considerable portion of the year in the GOA, which is critical when considering management and recovery plans for this endangered species. Timing of migrations to and from Alaskan waters is also important from a management perspective, especially considering these sperm whales interact with an important commercial fishery. We found no predictable timing of migration for the whales tagged in this study, indicating whales may leave the GOA heading south at any time of year. However, the sample size for this finding was small (nine tagged whales), and of the whales that left the GOA heading south, eight left between June and October, which is during the current longline fishing season from mid-March to mid-November. It is possible that while sperm whales may depart Alaskan waters heading south at any time of year, they are more likely to do so during certain seasons (summer and fall), making a case for shifting the timing of fishing seasons to reduce the effects of depredation. Thus, increasing the sample size of tag records, and targeting tagging efforts in the winter and spring may shed more light on the timing of migrations and when whales arrive and depart Alaskan waters.

Tagged whales dove almost continuously, often to the seafloor, where they were likely feeding on groundfish prey, including sablefish (Wild *et al.,* 2020). The proportion of sablefish in sperm whale diets has increased over recent decades (Wild *et al.,* 2020), during a time when sablefish biomass has generally decreased (Hanselman *et al.,* 2018b). Therefore, it is important for stock assessments to consider this additional source of sablefish mortality (both through depredation and potentially increased natural foraging) when assessing regional biomass of this commercially important species. In addition to sperm whale depredation as an additional source of mortality, killer whales also depredate sablefish from fishing gear in the GOA (Yano and Dahlheim, 1995), which has a greater impact on the annual stock assessment as well as the fleet than sperm whale depredation (Hanselman *et al.,* 2018b). Thus, the effects of removals from sperm whales and killer whales combined likely have a significant impact on the resource and incorporating all sources of mortality into assessments is important to determining sustainable harvest levels.

More broadly, our results identify a high potential for further conflict, as depredation may be a new and growing behavior facilitated by an overlap between natural foraging areas and the sablefish fishery in the GOA over the continental slope region. As sperm whale populations

increase world-wide, better understanding of their movement patterns in their GOA foraging grounds will be crucial to effectively protect fisheries resources and whale populations.

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3.7 Figures

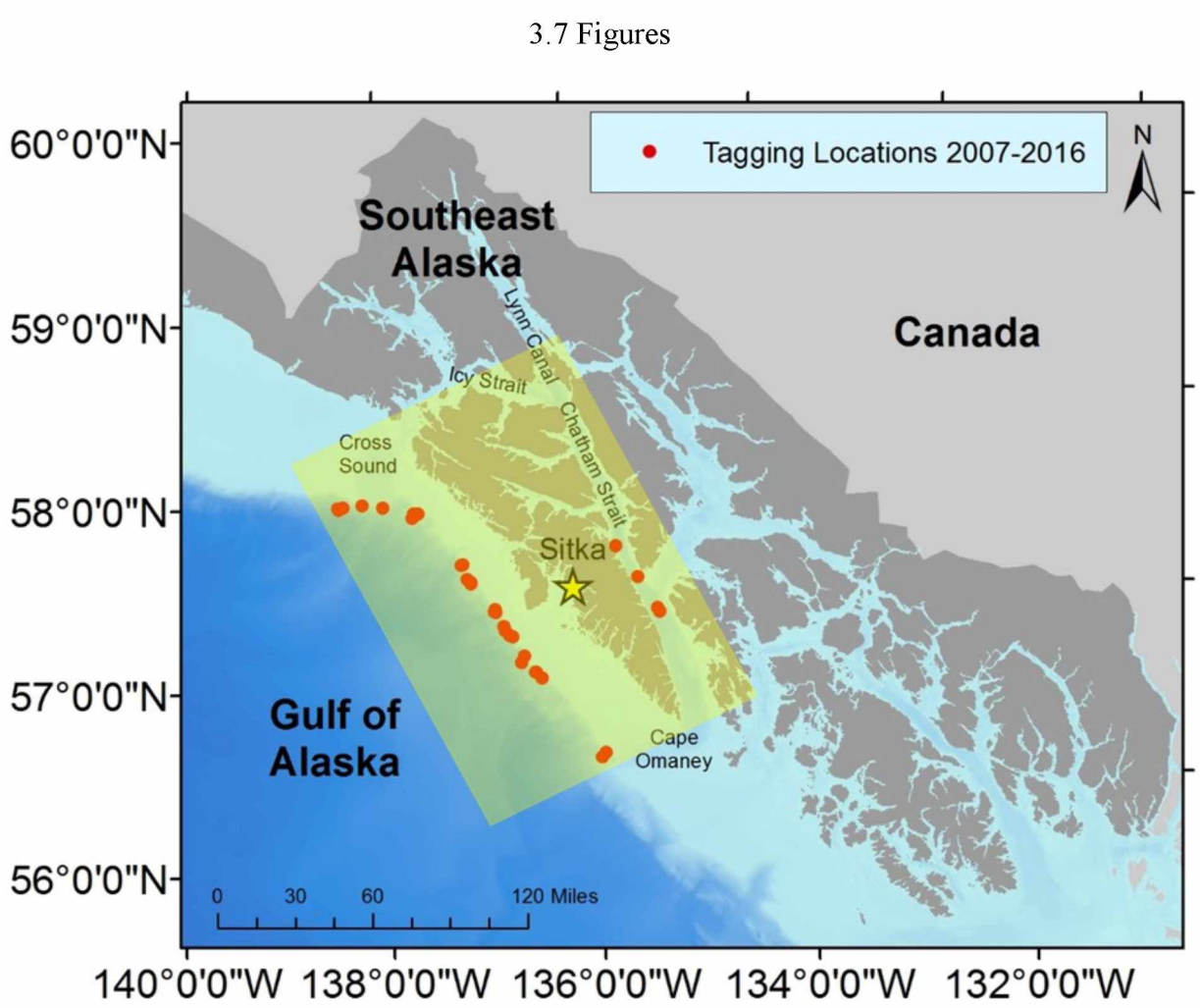

Figure 3.1 Locations, represented by red dots, where sperm whales (n=33 tags on 29 individuals) were tagged in the eastern Gulf of Alaska (GOA) study area, 2007-2016. The study area is depicted by the shaded box.

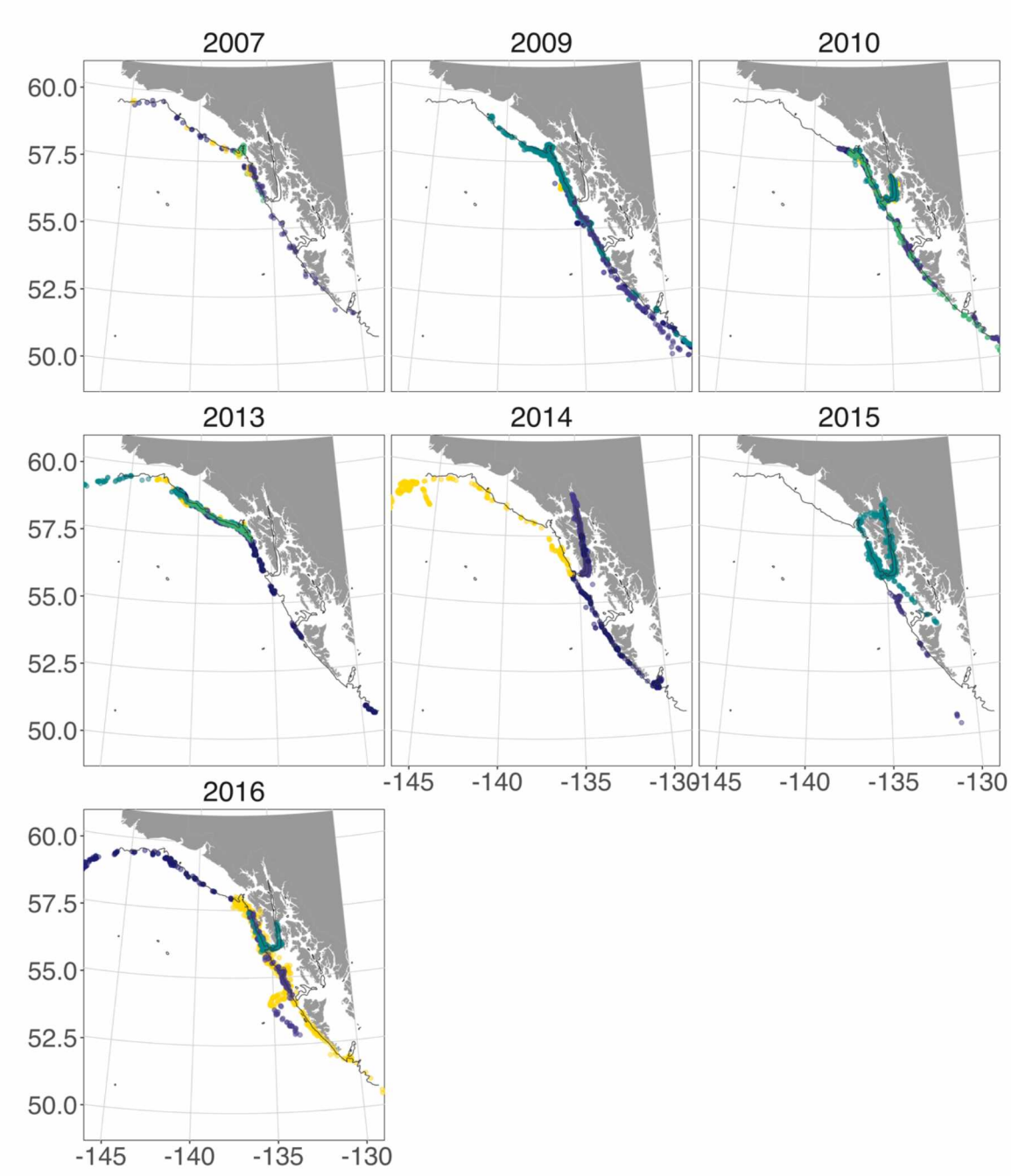

Figure 3.2 Satellite tag position estimates from filtered Argos data for all tags by year showing movement within the eastern Gulf of Alaska. The thin black line represents the bathymetric contour line at 350m depth. Different colors each year denote individual tagged whales. Full tag tracks are available in Supplemental Data S3.1.

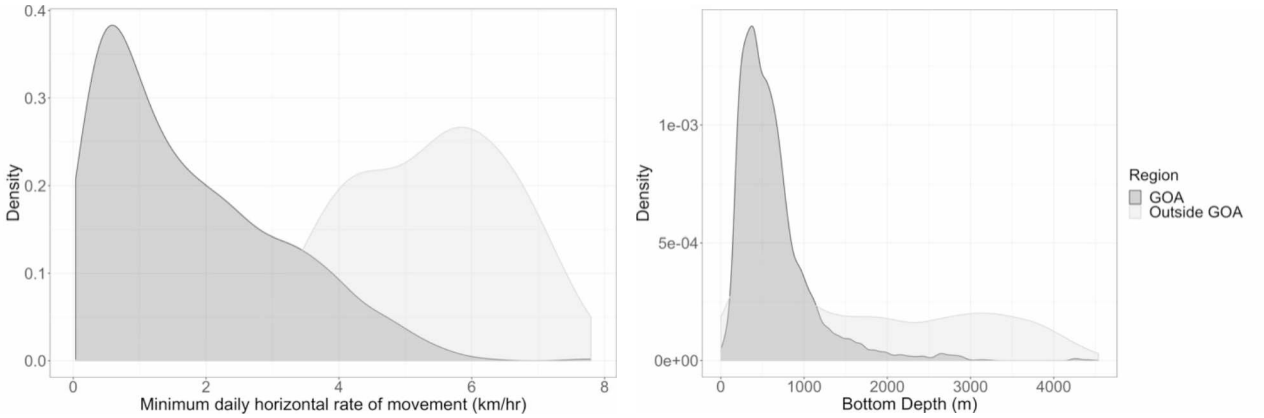

Figure 3.3 Density plot showing minimum rates of horizontal movement in the GOA versus outside the GOA. Whales have higher rates of movement after they leave the GOA.

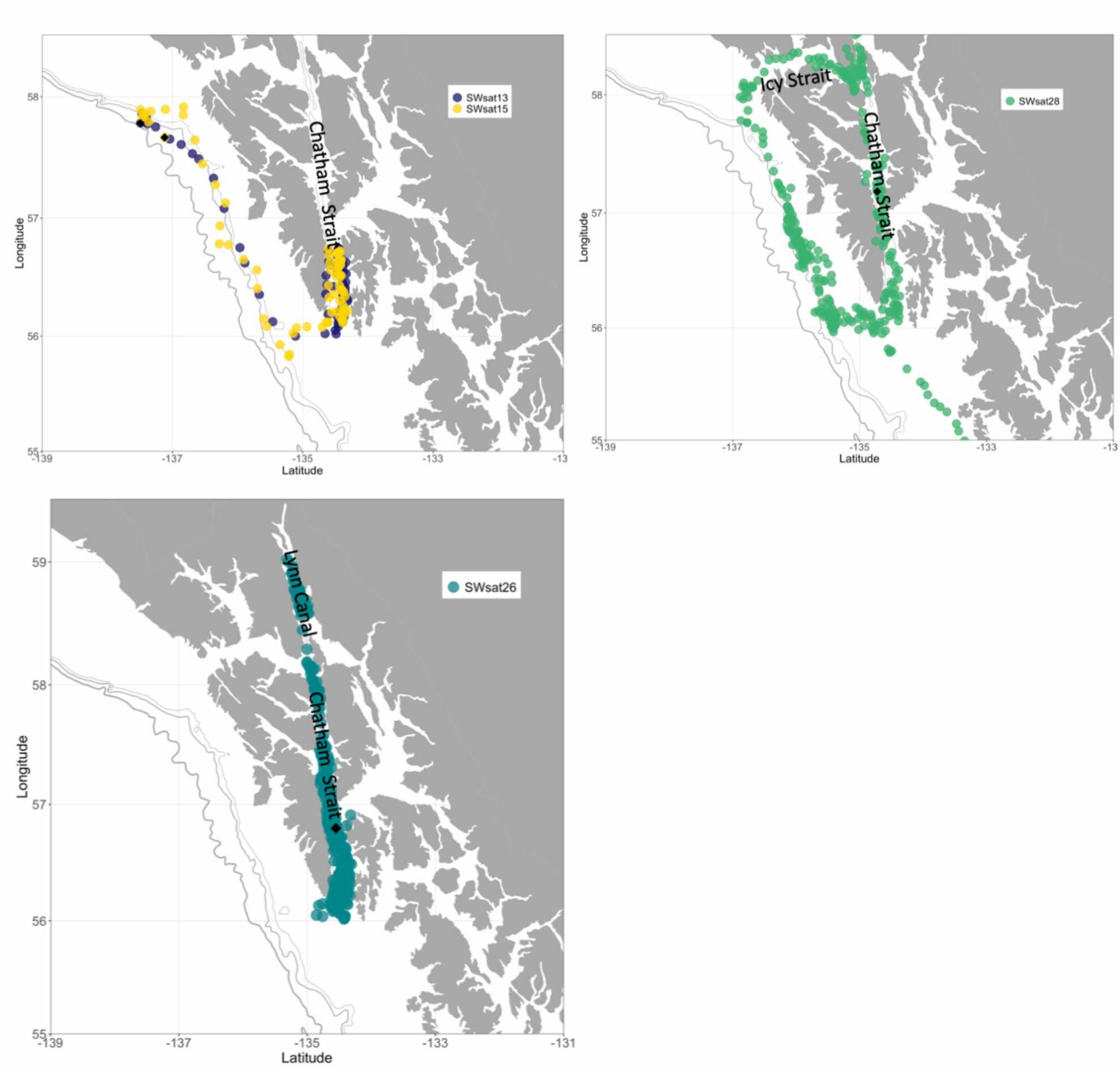

Figure 3.4 Tagged whales that entered Chatham Strait. Black diamonds denote tag attachment locations: a) Two individuals, SWsat13 (GOA-023) and SWsat15 (GOA-091), both tagged in early August, 2010, who moved into Chatham Strait concurrent with the state-managed sablefish fishery that opened in mid-August; b) SWsat28 (GOA-091), tagged in Chatham 2015, circumnavigated Baranof and Chichagof Islands ; c) SWsat26 (GOA-086), tagged in Chatham in 2014, moved north into Lynn Canal.

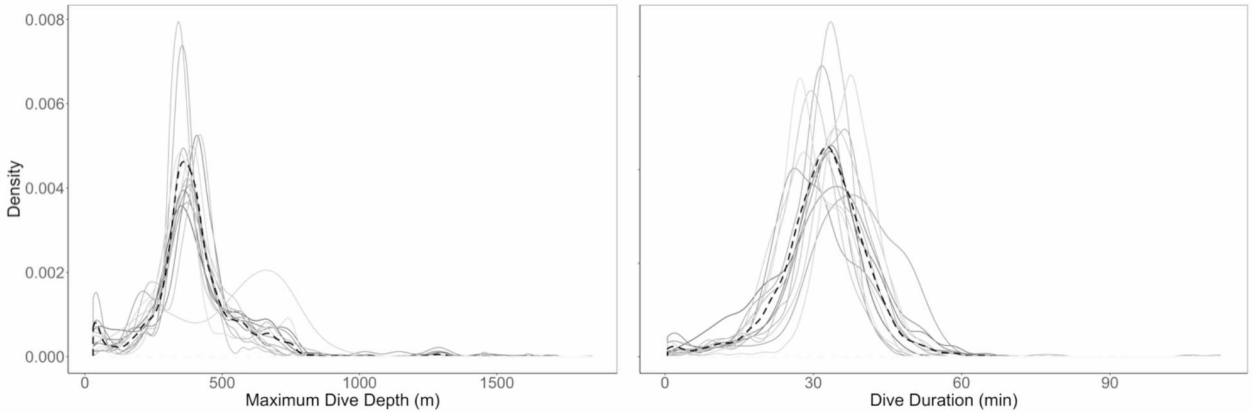

Figure 3.5 Density plots of maximum dive depth and dive duration for 14 tags with dive data. Dashed black line represents the average of all tags.

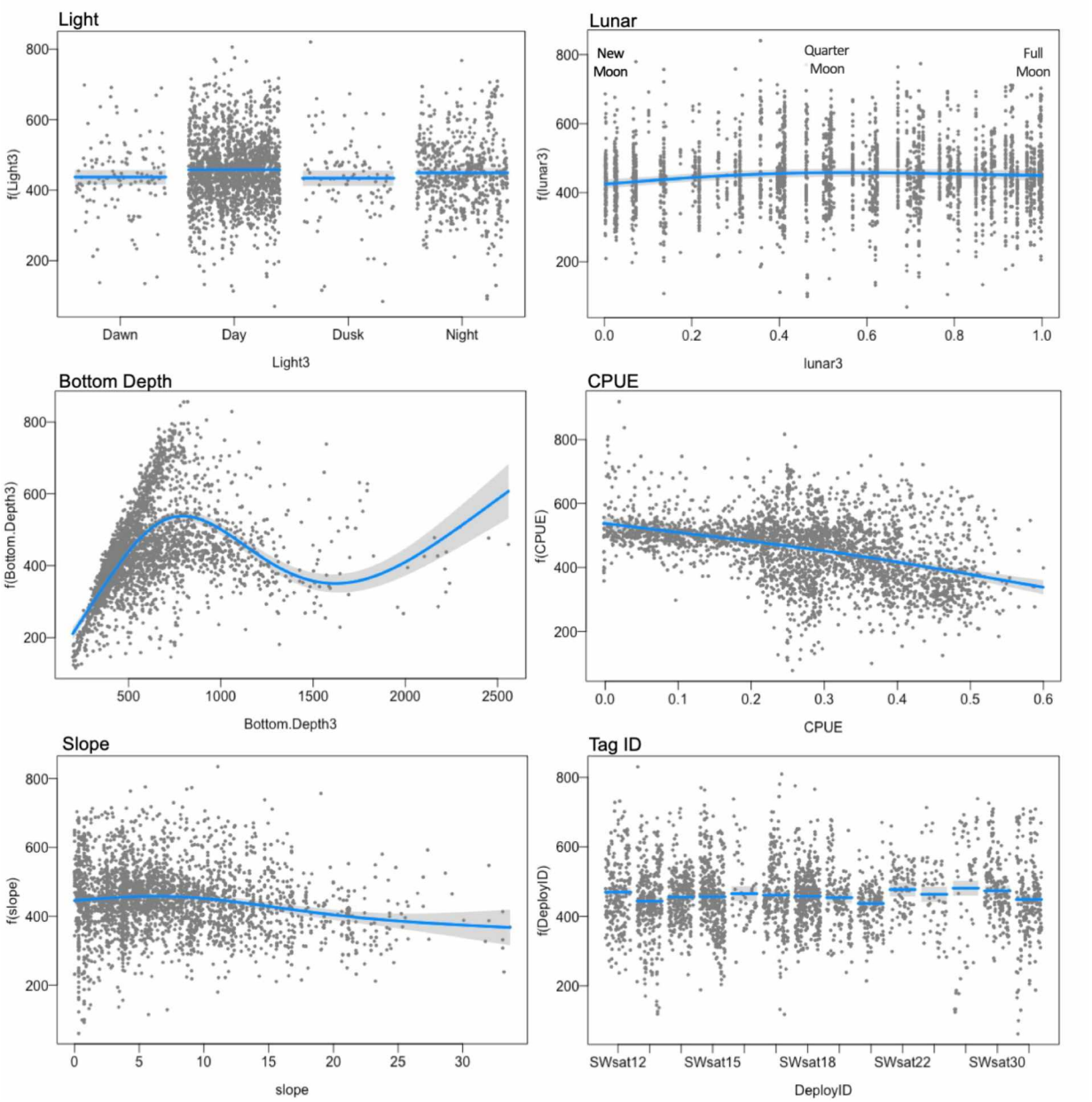

Figure 3.6 Model output of environmental covariates that influence dive depth, showing the smooth effect of each variable on dive depth (y-axis), conditional upon the other terms in the model at their median values. Blue line represents the prediction line with grey confidence band, and points represent partial residuals.

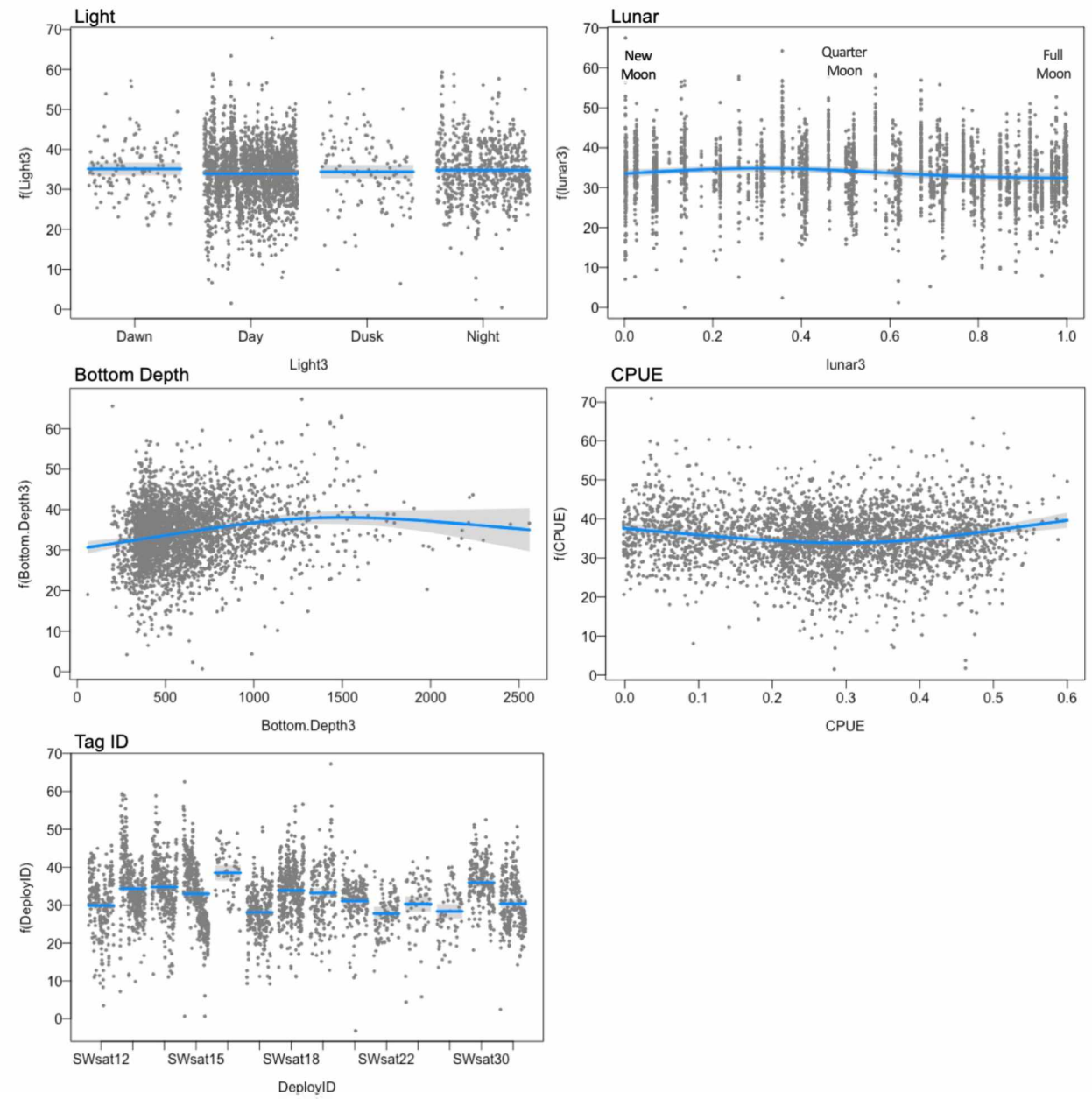

Figure 3.7 Environmental covariates from the best model for dive duration of sperm whales, showing the smooth effect of each variable on dive duration (y-axis), conditional upon the other terms in the model at their median values. Blue line represents the prediction line with grey confidence band, and points represent partial residuals.

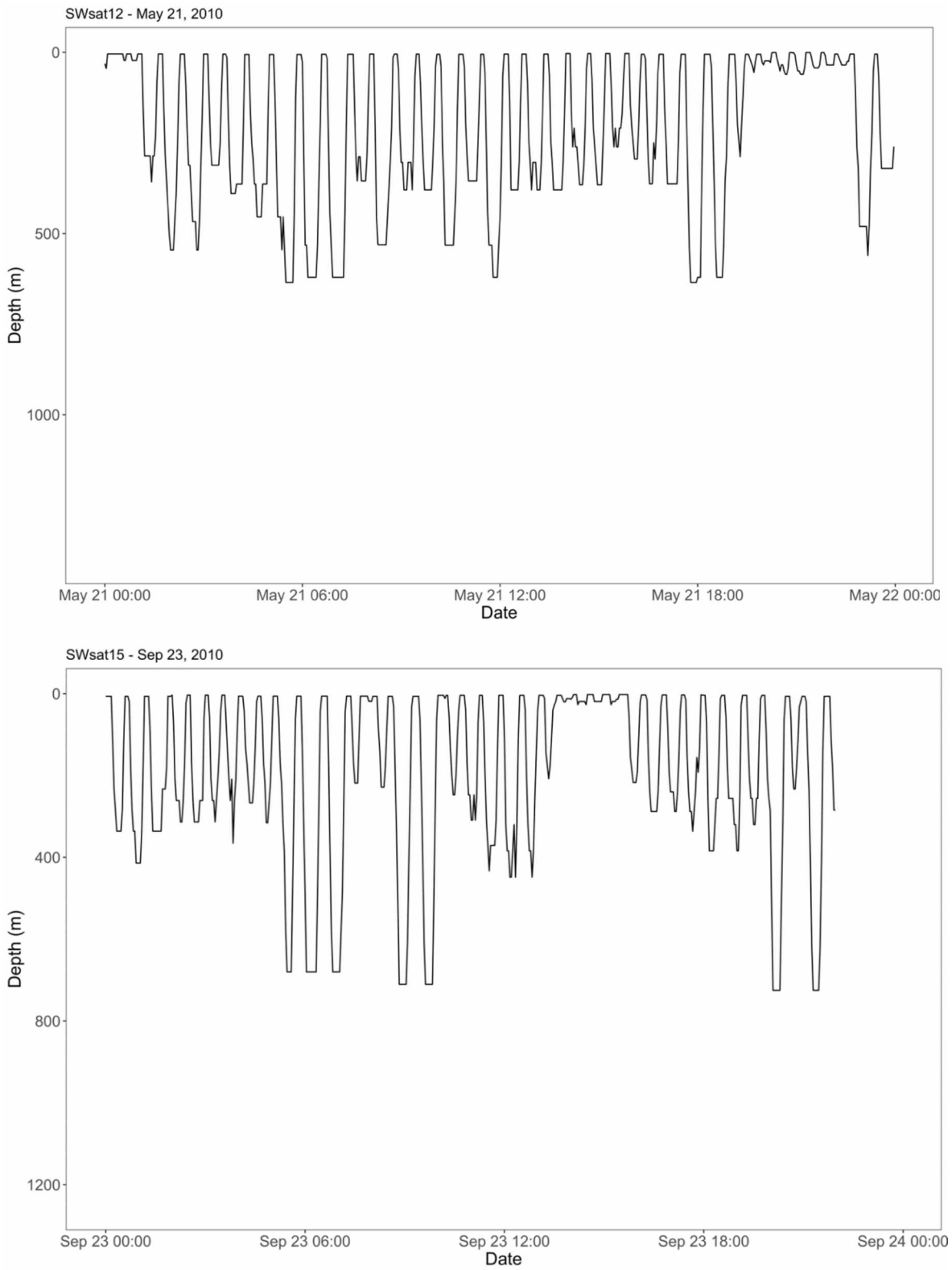

Figure 3.8 Time series data (low resolution) from two tags showing examples of switching between dive depths. Each plot shows a 24-hour day period.

3.8 Tables

Table 3.1 Summary of deployment data for 33 satellite tags placed on sperm whales by SEASWAP between 2007-2016. Four tagged whales were not photo-identified sufficiently for a unique identifier.

Year	Tag ID	Data Recorded	Date tagged (local)	Lat tagged	Lon tagged	# Days Transmit	Whale ID	Used in Analysis?
2007	SWsat1	Location-only	8-Jul-2007	57.027	-136.155	9	No ID	No[1]
2007	SWsat2	Location-only	8-Jul-2007	57.033	-136.175	34	GOA-068	Yes
2007	SWsat3	Location-only	14-Jul-2007	57.046	-136.161	3	GOA-047	No[1]
2007	SWsat4	Location-only	17-Jul-2007	56.873	-136.059	26	GOA-008	Yes
2007	SWsat5	Location-only	17-Jul-2007	56.884	-136.085	18	GOA-092	Yes
2007	SWsat6	Location-only	17-Jul-2007	56.912	-136.113	33	GOA-096	Yes
2009	SWsat7	Location-only	12-Jun-2009	56.606	-135.880	158	GOA-047	Yes
2009	SWsat8	Location-only	13-Jun-2009	56.647	-135.924	45	No ID	Yes
2009	SWsat9	Location-only	14-Jun-2009	56.750	-135.997	16	GOA-018	Yes
2009	SWsat10	Location-only	14-Jun-2009	56.646	-135.929	52	GOA-104	Yes
2009	SWsat11	Location-only	21-Jun-2009	56.721	-136.040	7	GOA-114	Yes
2010	SWsat12	Location + Depth	3-May-2010	57.331	-136.358	39	GOA-052	Yes
2010	SWsat13	Location + Depth	15-Aug-2010	57.795	-137.218	59	GOA-023	Yes
2010	SWsat14	Location + Depth	15-Aug-2010	57.805	-137.470	59	GOA-050	Yes
2010	SWsat15	Location + Depth	15-Aug-2010	57.812	-137.470	48	GOA-091	Yes
2010	SWsat16	Location + Depth	15-Aug-2010	57.811	-137.417	15	GOA-025	Yes
2013	SWsat17	Location + Depth	28-May-2013	57.330	-136.379	67	GOA-042	Yes
2013	SWsat18	Location + Depth	30-May-2013	57.671	-136.673	59	GOA-094	Yes
2013	SWsat19	Location-only	30-May-2013	57.655	-136.747	16	GOA-125	Yes
2013	SWsat20	Location + Depth	30-May-2013	57.679	-136.713	67	GOA-057	Yes
2013	SWsat21	Location + Depth	31-May-2013	57.755	-137.010	9	GOA-064	Yes
2014	SWsat22	Location + Depth	24-Jun-2014	56.092	-135.513	24	GOA-085	Yes
2014	SWsat23	Location + Depth	24-Jun-2014	56.081	-135.453	0	GOA-133	No[2]
2014	SWsat24	Location + Depth	25-Jun-2014	56.108	-135.466	72	GOA-133	Yes
2014	SWsat25	Location + Depth	25-Jun-2014	56.109	-135.481	27	GOA-050	No[3]
2014	SWsat26	Location-only	16-Sep-2014	56.782	-134.542	164	GOA-086	Yes
2014	SWsat27	Location + Depth	16-Sep-2014	56.804	-134.550	6	GOA-091	Yes
2015	SWsat28	Location-only	13-Sep-2015	57.200	-134.781	47	GOA-091	Yes
2016	SWsat29	Location-only	13-Jul-2016	56.939	-136.114	23	No ID	Yes
2016	SWsat30	Location + Depth	14-Jul-2016	57.217	-136.327	33	No ID	Yes
2016	SWsat31	Location-only	14-Jul-2016	57.225	-136.327	162	GOA-024	Yes
2016	SWsat32	Location-only	14-Jul-2016	57.248	-136.356	18	GOA-103	Yes
2016	SWsat33	Location + Depth	10-Sep-2016	57.000	-134.654	12	GOA-091	Yes

[1] Tag record contained fewer than 9 position estimates total and was deemed too short to provide useful information about the animal's movement and habitat use; [2] No transmissions received from the tag; [3]Tag did not successfully provide position estimates, likely due to the tag's placement being too low on the whale to successfully transmit more than one message to satellites on any satellite overpass, as required to obtain a position estimate.

Table 3.2 Summary statistics of dive information for each tag that transmitted diving behavior, showing mean and standard deviation (SD) for the maximum depth and duration of dives.

DeployID	Mean Max Depth (m)	SD Max Depth (m)	Mean Duration (min)	SD Duration (min)
SWsat12	387	173	29.2	9.18
SWsat13	422	220	33.9	9.24
SWsat14	405	154	33.2	10.85
SWsat15	406	186	30.1	9.74
SWsat16	418	214	37.8	9.80
SWsat17	379	156	29.9	6.83
SWsat18	387	149	34.0	7.82
SWsat20	401	168	32.4	10.62
SWsat21	350	118	31.7	6.87
SWsat22	385	139	28.9	6.82
SWsat24	378	120	34.8	8.19
SWsat27	507	222	29.9	6.94
SWsat30	405	116	37.0	7.53
SWsat33	369	160	29.4	6.97

Table 3.3 Summary of dive shape with respect to maximum dive depth, duration, and associated seafloor depth.

Shape	N	Mean Max Depth (m) (±SD)	Mean Duration (min) (±SD)	Seafloor Depth (m) (±SD)
Square	5401	394 (±135)	34 (±7)	586 (±386)
U	1757	422 (±224)	28 (±11)	640 (±398)
V	415	303 (±207)	28 (±13)	714 (±476)

Table 3.4 Summary output of dive depth and duration models. Note the slope parameter was dropped for the best model of dive duration. The last two columns show the proportion of deviance explained by each term when used as the single predictor and the additional variability explained by each term when added to a model that includes all other predictors.

Model	Predictors	F-value	p-value	Deviance explained (individually)	Deviance explained (with other terms)
Dive Depth	light level	4.57	0.003	0.002	0.003
	lunar cycle	8.65	< 0.001	0.019	0.006
	seafloor depth	376	< 0.001	0.223	0.240
	CPUE	115	< 0.001	0.059	0.095
	slope	20.3	<0.001	0.014	0.001
	Tag ID	3.31	< 0.001	0.058	0.012
Dive Duration	light level	3.12	0.025	0.002	0.003
	lunar cycle	8.45	< 0.001	0.015	0.001
	seafloor depth	39.6	< 0.001	0.051	0.027
	CPUE	21.1	< 0.001	0.041	0.020
	Tag ID	25.7	< 0.001	0.101	0.090

Table 3.5 Other studies including dive depth and durations around the world.

Study authors	Region (Sex)	Max dive depth	Dive durations	Methods
Watwood et al. 2006	Gulf of MX (F+M)	NR	45 +/- 6 min	DTAGs
Davis et al. 2007	Gulf of CA (F+M)	418 +/- 216 m	27 +/- 9 min	Satellite tags
Gallo-Reynoso et al. 2009	Gulf of CA (F+M)	342 +/- 196 m	23 +/- 13 min	Echosounders
Irvine et al. 2017	Gulf of CA (F+M)	325 +/- 239 m	25 +/- 14 min	Advanced Dive Behavior tags
Teloni et al. 2008	Norway (M)	492 +/- 593 m	32 +/- 10 min	DTAGs
Guerra et al. 2017	Kaikoura (M)	924 m	50 min	DTAGs
Jaquet et al. 2000	Kaikoura (M)	NR	41 min	Remote acoustics
This study	Gulf of Alaska (M)	396 +/- 116 m	32 +/- 9 min	Satellite tags

3.9 Appendices

A3.1 Dive depth data

Diving information was recorded and transmitted from a subset of 14 tags that were equipped with a pressor sensor. Tags transmitted dive information daily before switching to a duty cycle, depending upon the project objectives (Table A3.1.1). Start and end of dives were determined using a wet/dry sensor. Once the tag registered as 'wet', the tag had to cross a depth threshold of 30 m and last more than 30 sec before it was classified as a dive. If it did not exceed the 30 m depth threshold or 30 sec duration, the activity was classified as part of the surface interval. Depth was recorded at a resolution of 0.5 m for tags in 2010 (SWsat12-16) and 1 m for all other tags (2013-2016) (Table A3.1.1). Dive duration (T) was defined as the time spent below the 30 m qualifying depth or greater than the 30 sec qualifying duration. Duration of surface intervals was defined as the elapsed time between dives greater than the dive threshold qualifiers. The shape of dives was classified by the tag as either square, 'V', or 'U', and transmitted along with the other dive parameters. Assuming the bottom of a dive was any depth reading $\geq$ 80% of the maximum reading observed for the dive, bottom time (B) was defined as the time between the first bottom reading and the last bottom reading (Figure A3.1.1). The tag estimated B and T values internally and transmitted only the summary of a classification of dive shape for each dive. Shape was classified using the following parameters:

Square	$B > 50\%\ T$
V	$B \leq 20\%\ T$
U	$20\%\ T < B \leq 50\%\ T$

Finally, tags recording dive data also recorded and transmitted low-resolution time-series data, which was used to verify summary information and visualize tag data. Depth was read every 2.5min for a period of 24 hours. The time-series data was on a duty cycle of either starting with 1 day on before switching to a duty cycle of 3 or 5 days off and 1 day on or starting with 19 days on before switching to a duty cycle to 1 day off and 1 day on. SWsat12 was the exception to this, starting with 5 days on and then switching to a duty cycle of 1 day off and 1 day on. These data were recorded at a low resolution but could be used to show more approximate fine-scale movement during dives.

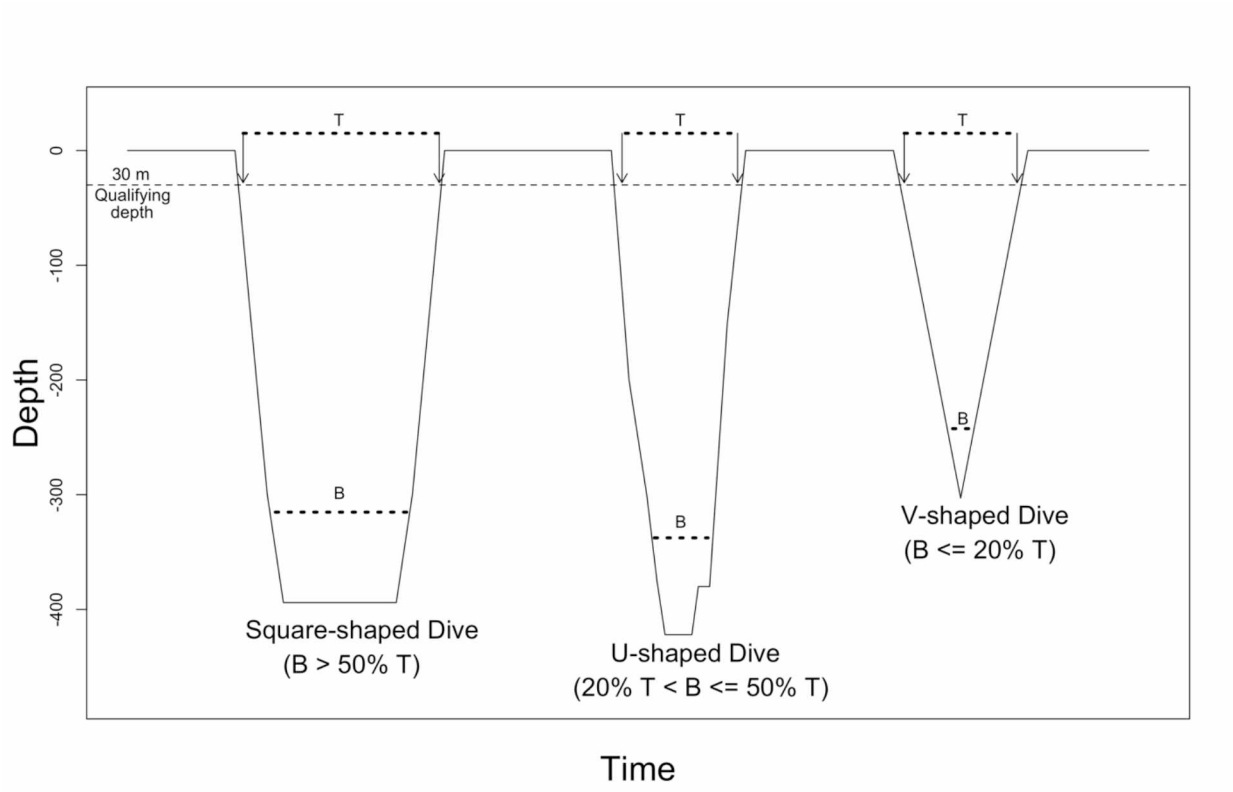

Figure A3.1.1 Classification of the three dive shapes. Bottom time (B) was calculated as the amount of time the tag was below 80% of the maximum dive depth for that dive. Total duration (T) was defined as the time below dive qualifying thresholds of 30 m or 30 sec. Varying dive depths and durations in the figure reflect the averages for each shape (Table 3 in manuscript).

Table A3.1.1 Summary of information recorded for each tag that recorded dive behavior information.

TagID	Date Tagged	Depth Sensor Resolution (Hz)	Collection Days: # Start	Collection Days: Duty Cycle	Collection Days = Transmit Days?	Transmit Days: # Start	Transmit Days: Duty Cycle	# Hours to transmit initially	Which hours to transmit each day
SWsat12	5/3/2010	0.5	5	Every other day for 360 days	N	20	Every other day for 10 days; Then every 10th day	1	0-7; 10-23
SWsat13	8/15/2010	0.5	19	Every 3rd day for 30 days; Then every 10th day	N	20	Every 3rd day for 30 days; Then every 10th day	1	0-7; 10-23
SWsat14	8/15/2010	0.5	19	Every 3rd day for 30 days; Then every 10th day	N	20	Every 3rd day for 30 days; Then every 10th day	1	0-7; 10-23
SWsat15	8/15/2010	0.5	19	Every 3rd day for 30 days; Then every 10th day	N	20	Every 3rd day for 30 days; Then every 10th day	1	0-7; 10-23
SWsat16	8/15/2010	0.5	19	Every 3rd day for 30 days; Then every 10th day	N	20	Every 3rd day for 30 days; Then every 10th day	1	0-7; 10-23
SWsat17	5/28/2013	1	20	Every 4th day for 24 days; Then every 8th day for 88 days	Y	20	Every 4th day for 24 days; Then every 8th day	24	0-7; 10-22
SWsat18	5/30/2013	1	20	Every 4th day for 24 days; Then every 8th day for 88 days	Y	20	Every 4th day for 24 days; Then every 8th day	24	0-7; 10-22
SWsat20	5/30/2013	1	20	Every 4th day for 24 days; Then every 8th day for 88 days	Y	20	Every 4th day for 24 days; Then every 8th day	24	0-7; 10-22
SWsat21	5/31/2013	1	20	Every 4th day for 24 days; Then every 8th day for 88 days	Y	20	Every 4th day for 24 days; Then every 8th day	24	0-7; 10-22
SWsat22	6/24/2014	1	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	Y	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	24	0-7; 10-22

SWsat23	6/24/2014	1	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	Y	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	24	0-7; 10-22
SWsat24	6/25/2014	1	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	Y	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	24	0-7; 10-22
SWsat25	6/25/2014	1	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	Y	19	Every 3rd day for 12 days; Then every 6th day for 30 days; then every 12th day	24	0-7; 10-22
Swsat27	9/16/2014	1	19	Every 3rd day for 12 days; Then every 10th day	Y	19	Every 3rd day for 18 days; Then every 6 days for 48 days; then every 12th day	48	1-7; 9-23
SWsat30	7/14/2016	1	80	Every 3rd day for 12 days; Then every 10th day	Y	80	Every 3rd day for 12 days; Then every 10th day	24	0-7; 10-23
SWsat33	9/10/2016	1	39	Every 3rd day for 12 days; Then every 10th day	Y	39	Every 3rd day for 12 days; Then every 10th day	24	5-8; 11-23

A3.2 Sablefish catch data

To assess sablefish catch-per-unit-effort (CPUE) with respect to whale movement, we used data collected from observers on longline fishing vessels participating in the sablefish fishery in the Southeast (SE) statistical area of the GOA, with permission from the NOAA Fisheries' Ted Stevens Marine Research Institute (TSMRI) in Juneau, Alaska. These data do not include catch data from inside waters (e.g. Chatham Strait), where a separate sablefish fishery is managed by the Alaska Department of Fish and Game. Catch data was collected between 1995 and 2019. We calculated a CPUE index for each sablefish longline set in the database as the logarithm of catch per unit of effort (kg / 1000 hooks + 1) (Mateo and Hanselman, 2014). Additional covariates for each set included year, date, bottom depth, set location, and vessel length. For set location we calculated the mid-point of each set from the start and end locations.

To quantify the average spatial pattern of sablefish CPUE for use in the behavioral model we modeled CPUE as a smooth function of latitude and longitude using a generalized additive mixed effect model (GAMM) of the form:

$$CPUE = \alpha + a_y + f_1(lat, lon) + f_2(depth) + f_3(day) + f_4(V.length) + \varepsilon$$

where α is the intercept, a_y is a random intercept for year y to account for within-year correlations and differences in average catch rates among years, f_1 is a smooth bivariate function of latitude and longitude and f_2-f_4 are smooth univariate functions of bottom depth (*depth*), day-of-the-year (*day*) and vessel length (*V.length*). The residuals (ε) are assumed to follow a spatially autocorrelated random process with a Gaussian correlation structure to account for any remaining within-year spatial autocorrelation. The model was fit via restricted maximum likelihood assuming a Gaussian distribution and smoothing parameters were estimated using the generalized cross validation criterion (GCV) to reduce the likelihood of over-fitting (Wood, 2017). Our 'base model' assumes the spatial pattern of CPUE is consistent from year to year, which we tested by comparing the base model with two alternative models. The first model fit separate smooth surfaces of CPUE by year ($f_{1,y}(lat,lon)$) and a second alternative fit CPUE for two separate time periods before and after tagging data were available for sperm whales (i.e. before 2007 and from 2007 to present). The model with year-specific smooth functions did not converge due to large spatial gaps in the data for individual years. Therefore, we fit separate

models by year and visually compared the estimated surfaces among years. All models were fit in the package "mgcv" in R (R Core Team, 2019). Model selection was carried out using residual plots and the Akaike information criterion (AIC) to compare reduced models consisting of all possible subsets of the terms f_2-f_4.

The full model was used to predict sablefish CPUE on a 0.02 decimal degree grid (approximately 1 x 1 nautical mile) spanning the observed fishing sets. The gridded area encompassed most of the recorded sperm whale foraging locations in the study area (75%). Depth at the center of each grid cell was obtained by matching its location to the bathymetric surfaces in ArcGIS. Sablefish CPUE was then predicted based on the latitude, longitude and depth of each grid point, with the values for vessel length and Day of the Year fixed at their medians (66 ft and day 137, respectively, corresponding to the typical size of most longline fishing vessels in the Southeast region and to the midpoint of fishing effort during the longline fishing season). We arbitrarily selected 2016 to visualize the spatial pattern but ran predictions over a variety of years and saw very little variation in the range, mean, and median CPUE predictions for the gridded area (Figure A3.2.1).

The best model indicated that sablefish CPUE peaked between 57° and 57.5° N Latitude, and -136° to -137° W Longitude. Sablefish CPUE increased as bottom depth increased and decreased as vessel length increased Figure 3.B2). There was a seasonal variability in sablefish CPUE, with decreasing CPUE from the beginning of the season until late April, and then increasing again through the summer months. Finally, CPUE has been decreasing by year since 1995 (Figure A3.2.2).

References:

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R Core Team. 2019. R: A language and environment for statistical comuting. R Foundation for Statistical Computing, Vienna, Australia.

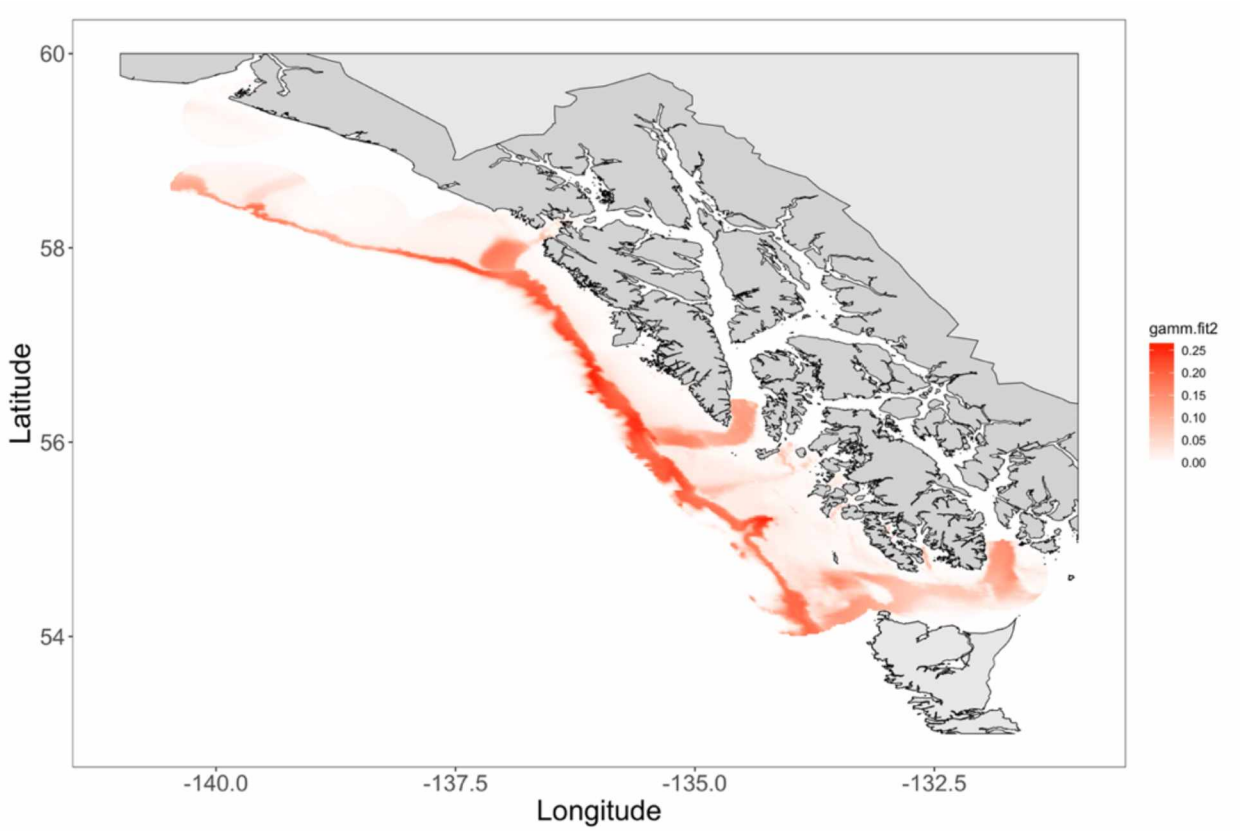

Figure A3.2.1 Predicted sablefish CPUE from modeled observer catch data in the Southeast (SE) statistical area of the GOA. NOTE: gamm.fit2 is the CPUE.

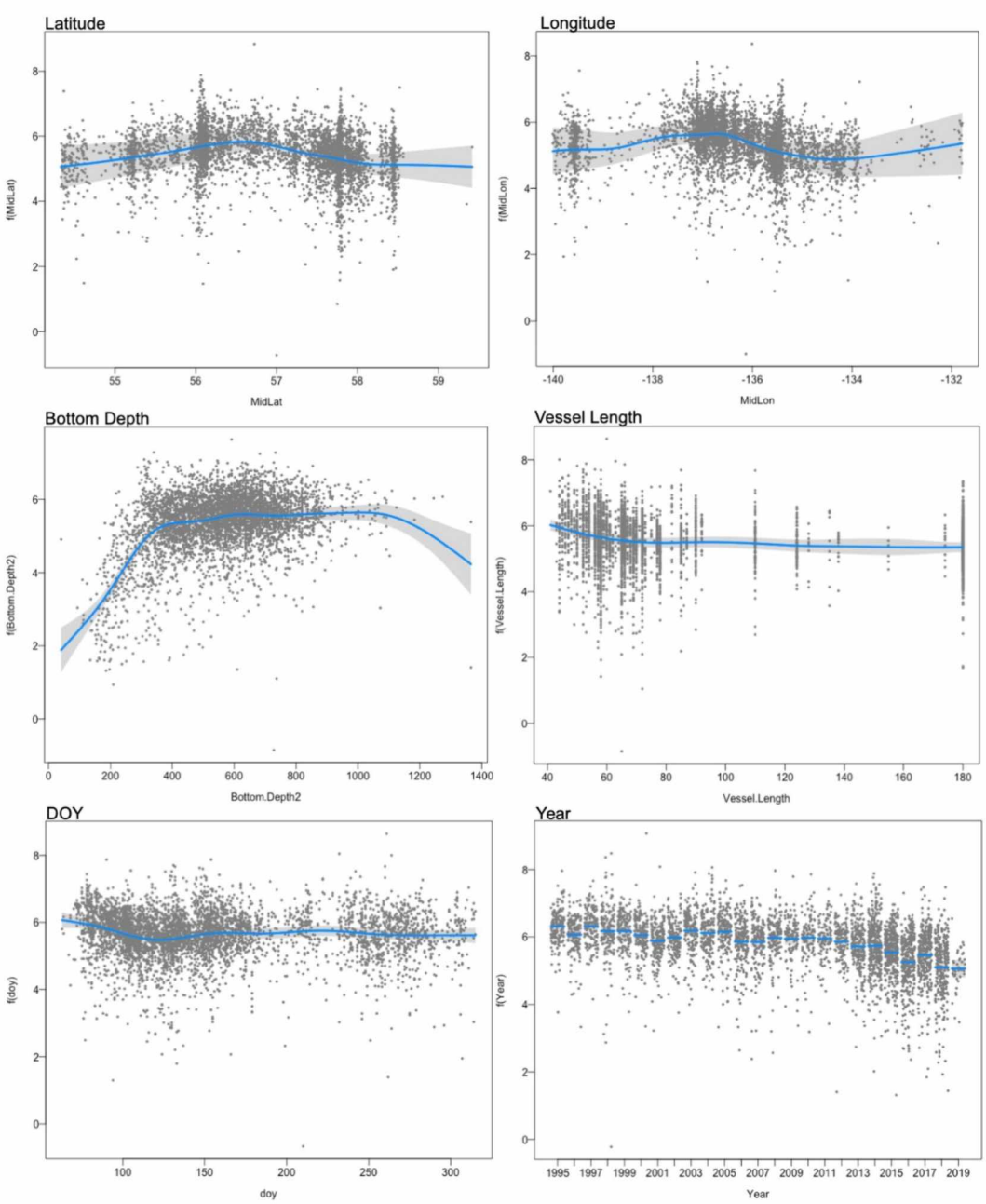

Figure A3.2.2 Gamm output showing how CPUE changes with respect to location (Latitude & Longitude), seafloor depth, vessel length, day of the year (DOY), and Year.

A3.3 Interannual comparison of the dive behavior of an individual whale

A single individual sperm whale (GOA-091) was tagged in multiple years, as SWsat15 in 2010, SWsat27 in 2014, SWsat28 in 2015, and SWsat33 in 2016 (Figure A3.3.1). Tags transmitted 48, 6, 47, and 12 days respectively. SWsat15 was deployed in August 2010, while SWsat27, SWsat28, and SWsat33 were all deployed in mid-September 2014, 2015, and 2016 respectively.

Tagging location & general movement:

In 2010, GOA-091 was first tagged by SEASWAP, offshore in the eastern GOA in an area referred to as the Spencer Spit. In all other years (2014-2016), GOA-091 was tagged in inside waters of Chatham Strait, in Southeast Alaska. This individual was identified in Chatham during the sablefish fishery across multiple years during a SEASWAP tagging project targeting sperm whales in Chatham during those years (2014-2016). However, even in 2010, the year GOA-091 was tagged offshore, the whale moved into Chatham Strait while the tag was transmitting, suggesting that this individual frequents this habitat during the fall months. In 2015 the whale circumnavigated Baranof and Chichagof Islands, going through Icy Strait, a fairly narrow passage that becomes shallow (< 300m) in places (Figure A3.3.1). None of the tags stayed on the individual longer than 50 days, so location information was fairly localized around the study area and Chatham Strait. During deployment SWsat28, the individual moved onto the shelf while migrating south, ending off the Northeast tip of Haida Gwaii in British Columbia at the end of the deployment, which was further inshore than any other tagged animal (Figure A3.3.1, Figure 3.3). It is not unheard of for a sperm whale to be in this region, as historical whaling data show that a few male sperm whales were taken north of Haida Gwaii in the mid-1900s (Gregr, 1992).

Dive behavior:

This animal was tagged with SPLASH tags that transmitted diving behavior data in three of the four years it was tagged (all years except 2015, SWsat28). Mean (±SD) maximum dive depth varied from 406 m (± 186 m) in 2010 (SWsat15) to 507 m (± 223 m) in 2014 (SWsat27) and 369 m (± 160 m) in 2016 (SWsat33) (Figure 3.C2, Table 3.C1). Dive duration was very similar, averaging 30 min (± 10 min) in 2010, 30 min (± 7 min) in 2014, and 29 min (± 7 min) in 2016 (Figure A3.3.2, Table A3.3.1). Mean dive depths of this one individual span most of the range of mean dive depths observed in this study (350 m to 507 m, Table 2), whereas mean dive depth

was much more consistent compared to the full range of observed mean dive durations (28.9 to 37.8 min).

Dive shape reflected the pattern shown in all tags, with a majority of square-shaped dives, followed by U-shaped dives, and the fewest V-shaped dives (Table A3.3.2).

Site fidelity:

GOA-091 appears to exhibit site fidelity to Chatham Strait during the fall season; this individual was tagged in Chatham Strait on three occasions and entered the Chatham Strait region when tagged a fourth time in outside waters. However, this result represents a small range of deployment duration (n = 6 to 49 days) and a small number of years (n=4). In addition, tagging effort was focused for three of the years within the Chatham Strait region so the full range of movement is biased toward that region.

References:

Gregr, E. J. 1992. An analysis of historic (1908-1967) whaling records from British Columbia, Canada.

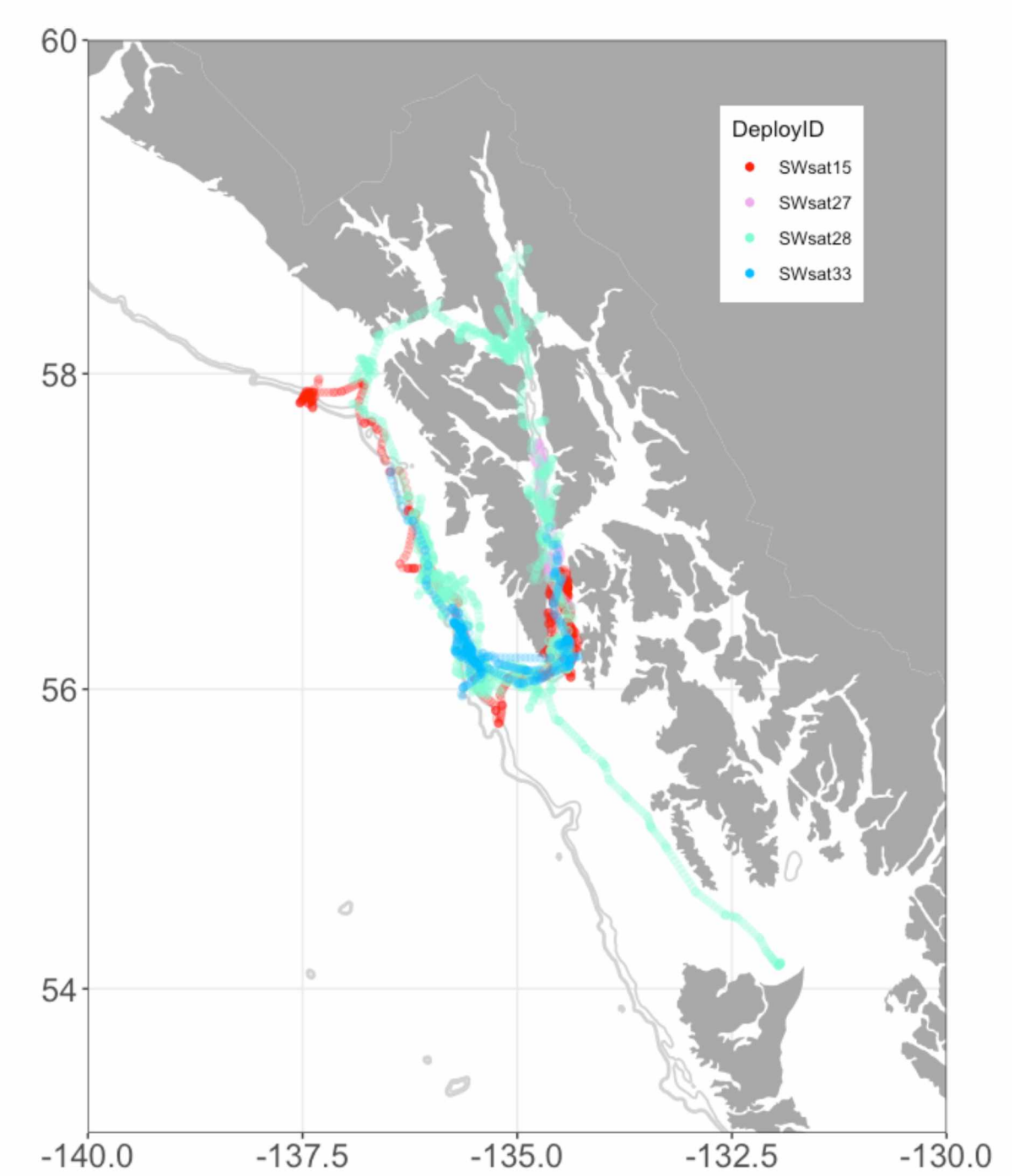

Figure A3.3.1 Map of the eastern GOA showing tag tracks for each time GOA-091 was tagged. Tracks show interpolated hourly positions from state-space models.

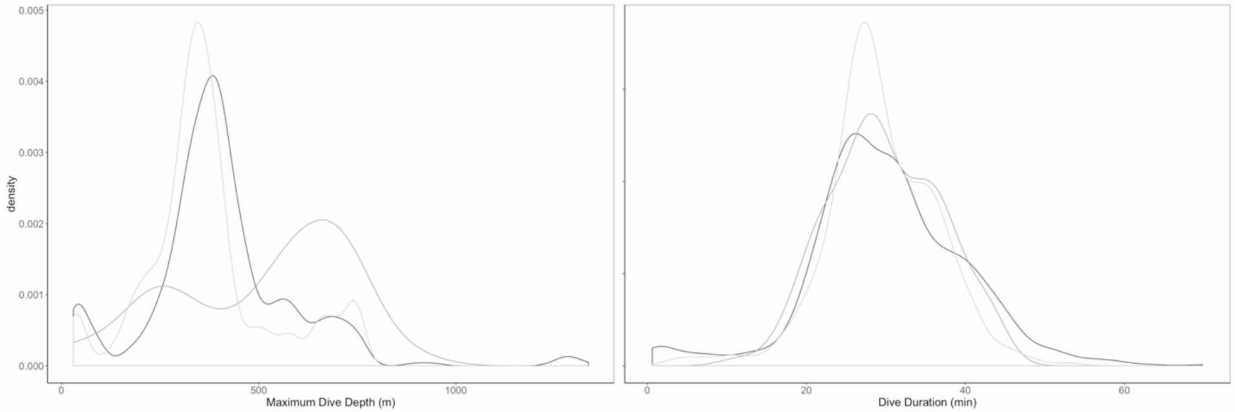

Figure A3.3.2 Density plots of each of SWsat15, SWsat27, and SWsat33's dive depth and durations.

Table A3.3.1 Average maximum dive depth and dive duration (±SD) for GOA-091's three tag deployments with dive information.

Deploy ID	Max Dive Depth (±SD) (m)	Dive Duration (±SD) (min)
SWsat15	406 (186)	30 (10)
SWsat27	507 (223)	30 (7)
SWsat33	369 (160)	29 (7)

Table A3.3.2 Dive shape of GOA-091's dives from SWsat15, SWsat27, and SWsat33.

Deploy ID	Square-shaped	U-shaped	V-shaped
SWsat15	597	293	63
SWsat27	54	33	18
SWsat33	302	93	21
TOTAL	**953**	**419**	**102**

A3.4 Proportion of the water column used by tagged whales

In the overall data set, 6373 interpolated locations had associated dives. Of these, 2359 (37%) had dive depths *deeper* than the seafloor depth, while the remaining 4015 (63%) had dive depths shallower than the seafloor depth. For dive depths that were deeper than seafloor depths, the calculated distance between the maximum dive depth and seafloor depth was negative (Figure A3.4.1).

The proportion of the water column whales dove in was calculated, and twenty-three outliers were removed, for which the proportion of the water column whales were diving in was greater than 10 (1000%) (Figure A3.4.2). These data points were most likely where the seafloor depth measured close to 0 m, due to variability in accuracy of interpolated locations as well as resolution of seafloor depth calculations. The estimated proportion of the water column whales dove to ranged from 0.02 (2%) of the water column to 9.7 (970%) of the water column. [Proportions from 0-1 are from whales whose maximum dive depth was *less than* the seafloor depth, and proportions 1-9.7 are from whales whose maximum dive depth was *greater than* the seafloor depth associated with the estimated hourly location]. Assuming the assigned seafloor depth is as likely to be overestimated as underestimated from the true seafloor depth (Figure A3.4.1), the median ratio of dive depth to seafloor depth should give a reasonable estimate of the central tendency for the proportion of the water column used by whales. The median proportion of the water column used by whales in this data set was 0.84, or 84% of the water column.

We subset the data to isolate just the dives in which the maximum dive depth was less than the seafloor depth (63% of data points), and visually examined them for patterns and variability (Figure A3.4.3).

We then zoomed in on specific tags and days to explore how close to the seafloor whales dove and how that changed over a smaller time period (Figure A3.4.4). A times, dives appeared to follow the bathymetry, while other times the bathymetry changed but the dive depths did not, or vice versa (Figure A3.4.4). These instances are likely due to the error inherent in the data and our methods:

1) Location error estimated from satellite tags
2) Modelling error inherent in interpolating hourly tag positions
3) Error from match-up of tag dives to hourly locations based on date/time stamp. [5 minutes subtracted to beginning and added to end of each dive; hourly tag estimates that fit within that time were given those dive characteristics].
4) Bathymetry estimation error from lower resolution bathymetric mapping.

Alternatively, these changes could be due to shifting prey that whales follow, using knowledge gained from previous dives. Interpretation of these data should be taken carefully as the consecutive estimates of seafloor depth are strongly autocorrelated. Because locations are smoothed by the models and because the continental slope is a relatively linear feature, if the model "strays" from the true depth into shallower or deeper areas, it is likely to stay there for a while, which could lead to some of the patterns observed (i.e. seafloor depths moving away from dive depths and then back towards them) (Figure A3.4.4).

These data show that whales often dive to the seafloor, and also sometimes don't dive to the seafloor. This matches with some preliminary SEASWAP data, which shows that acoustically, whales were diving mid-water, and not to the seafloor, while naturally foraging (Thode *et al.*, 2006). Caution must be taken not to over-interpret these plots, due to uncertainty in the seafloor depth estimates, and autocorrelation of the simulated locations used to extract depths. Nonetheless we can assume sperm whale diving to be complex, with whales targeting a variety of depths and portions of the water column, with decisions about dive depth to be made based on environmental variables (e.g. bathymetry, currents, etc.) as well as based upon knowledge from the previous dive regarding prey availability. While sperm whales in this region clearly dive to the seafloor often, they also spend time performing dives that likely do not take them to the seafloor, but instead to another mid-water depth for reasons perhaps due to prey detection, or to target a depth where sound transmits well (i.e. the SOFAR channel), or simply for transiting purposes.

References:

Thode, A. M., Straley, J., Tiemann, C., Teloni, V., Folkert, K., O'Connell, V., and Behnken, L. 2006. Sperm Whale and Longline Fisheries Interactions in the Gulf of Alaska - Passive Acoustic Component 2004. 56pp pp.

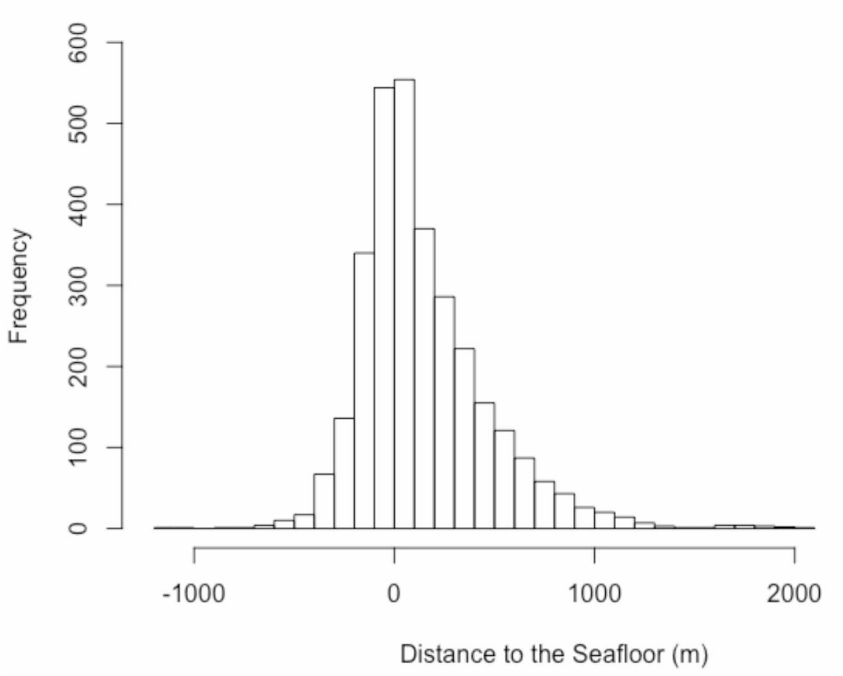

Figure A3.4.1 Histogram of the distance between the maximum depth for a dive and the seafloor depth.

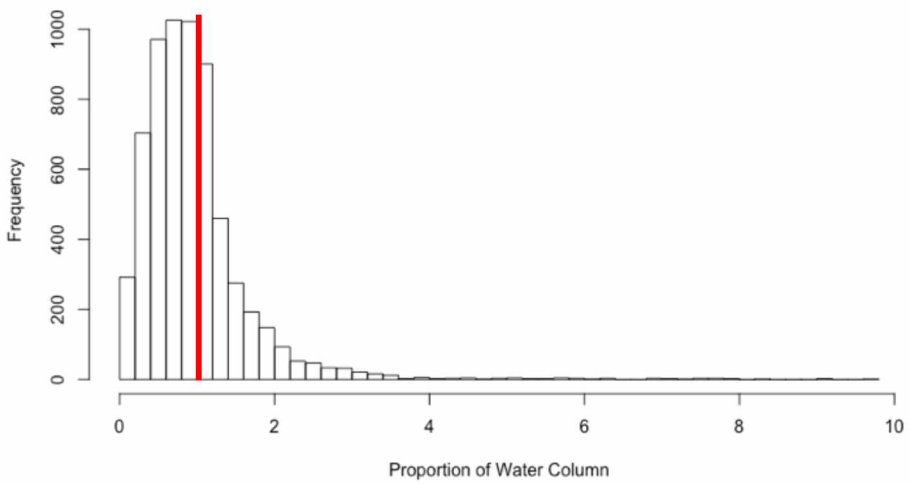

Figure A3.4.2 Proportion of water column whales dove to. Dives from 0-1 were those in which the maximum dive depth was less than the assigned seafloor depth. Red line indicates the break between dive depth less than seafloor depth (left of the line) and dive depth greater than seafloor depth (right of red line).

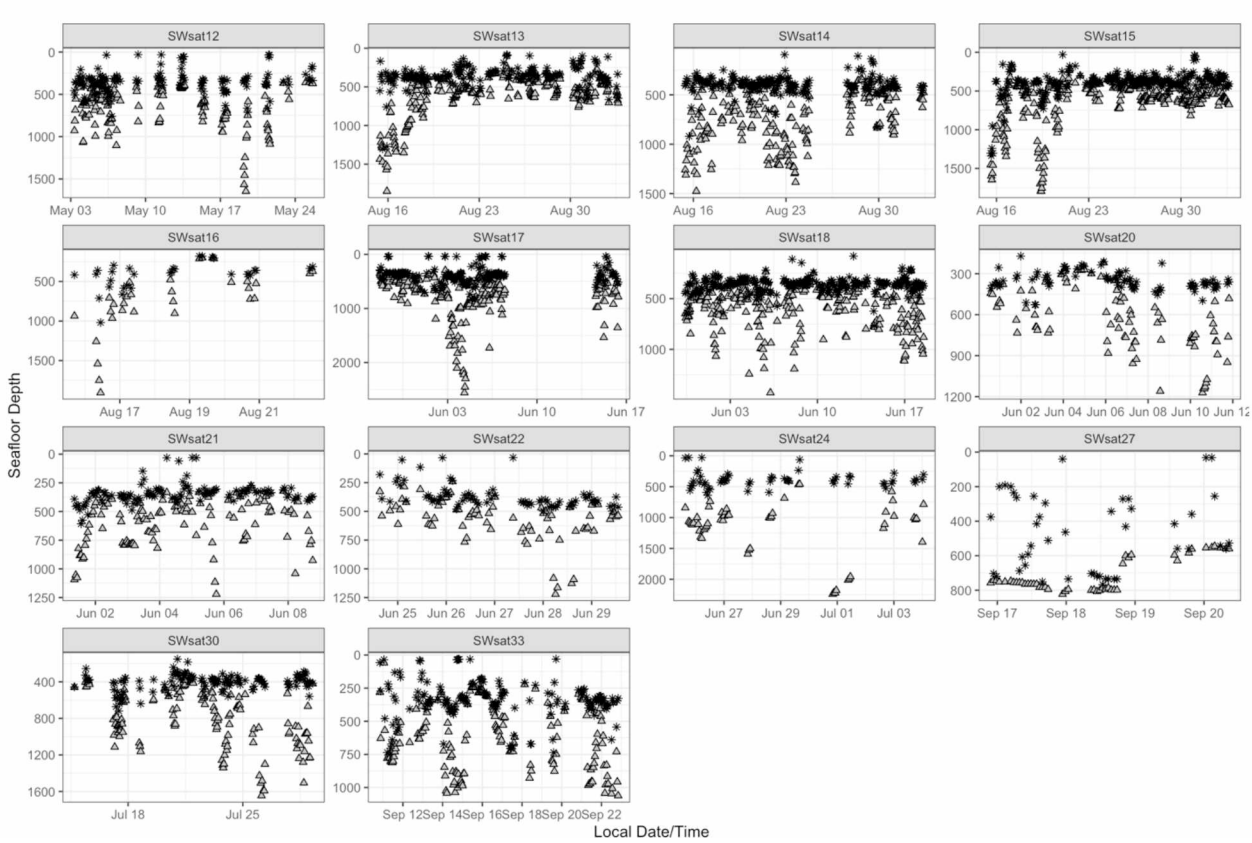

Figure A3.4.3 Maximum dive depths (asterisks) plotted over the seafloor depth (grey triangles) for the corresponding approximate location, for subset of data where dive depth < seafloor depth.

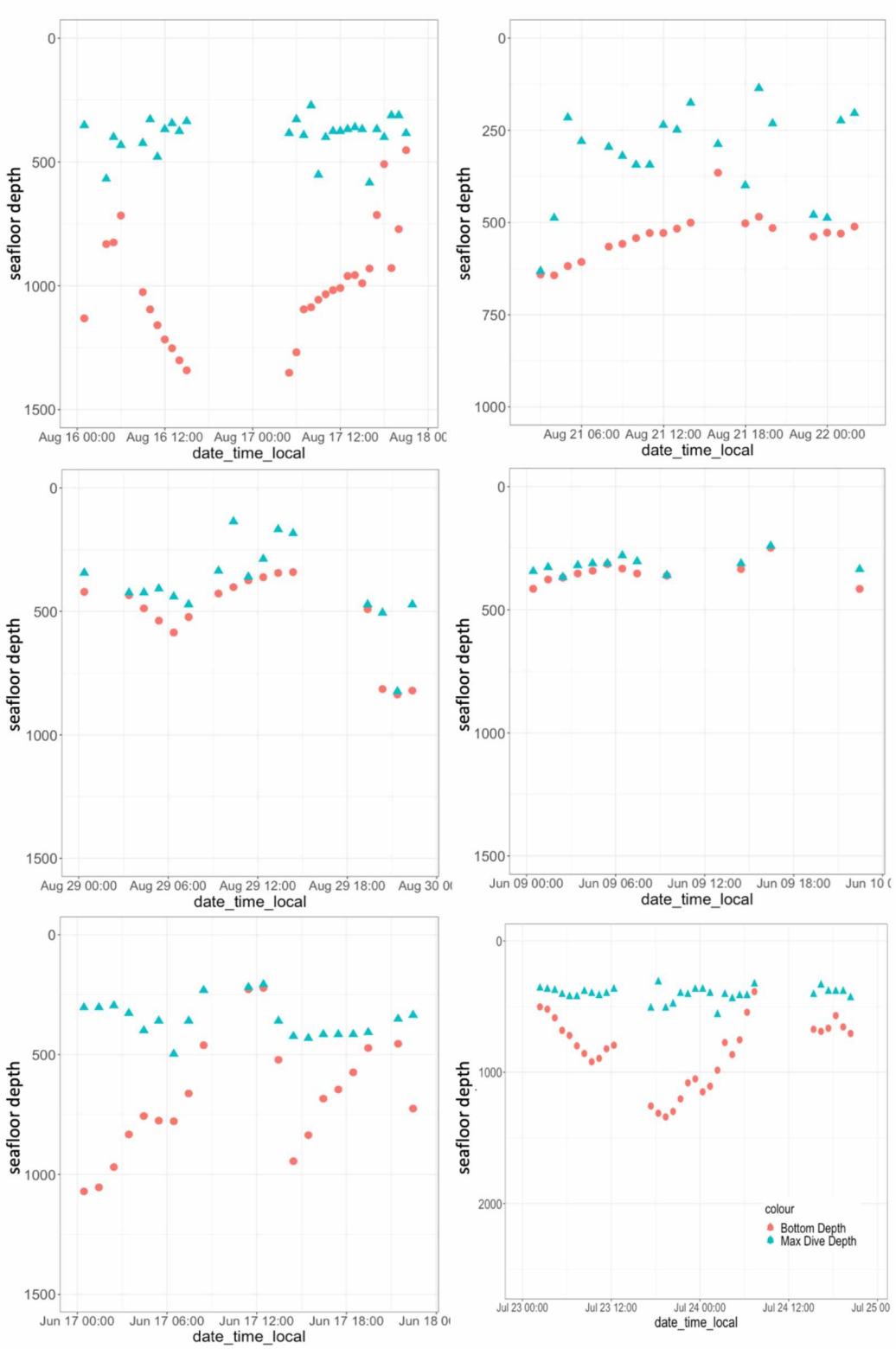

Figure A3.4.4 Individual dive plots showing the seafloor depth (red circle) and maximum dive depth (blue triangle) for selected time periods. Plots illustrate whale proximity to the seafloor when diving.

A3.5 Dive shape analysis

The shape of dives was estimated as Square, U, or V shaped, and transmitted in messages when the tag was at the surface, as described in Appendix A3.1. Statistical analyses with this data set would be difficult because the data are highly autocorrelated, but we looked for visual patterns in the data below. While there was some variability among individuals in the proportion of each dive type, tagged whales in this study primarily exhibited Square-shaped dives, followed by U-shaped dives, and very few V-shaped dives (Figure A3.5.1).

The maximum dive depth, duration, and seafloor depth associated with the dive for each dive type did vary both within and among individuals (Figures A3.5.2, A3.5.3, & A3.5.4). V-shaped dives were often both shallower and shorter than the other two dive types (Figures A3.5.2 & A3.5.4). Square-shaped dives had the most consistent depths among individuals (Figure A3.5.2).

We assessed dive shape with respect to light levels and lunar cycle to explore potential functional differences between dive types (Tables A3.5.1 & A3.5.2). The percentage of square, U, and V-shaped dives with respect to light levels was fairly similar, though daytime showed a higher percentage of square-shaped dives than other time periods, and than U or V-shaped dives (Table A3.5.1). New moons had the highest percentage of Square-shaped dives, though there was generally little variability in dive shape with respect to lunar cycles as well.

Seafloor depths associated with each dive shape did vary considerably (Table A3.5.3). Square-shaped dives were typically performed in shallower water, while V-shaped dives were performed in the deepest water (Table A3.5.3). This result is interesting because V-shaped dives also had the smallest dive depth (Table A3.5.3).

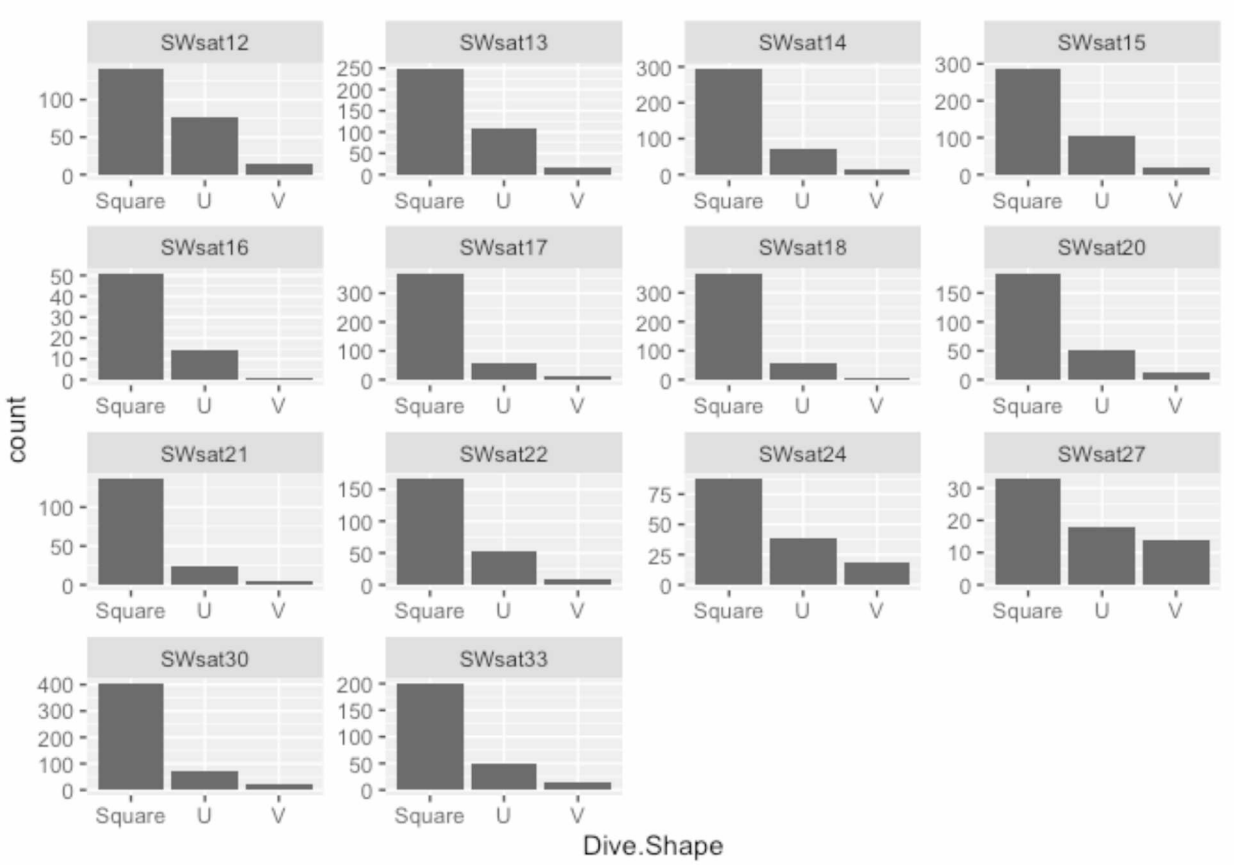

Figure A3.5.1 Quantity of each dive type displayed by each tagged whale that collected dive information.

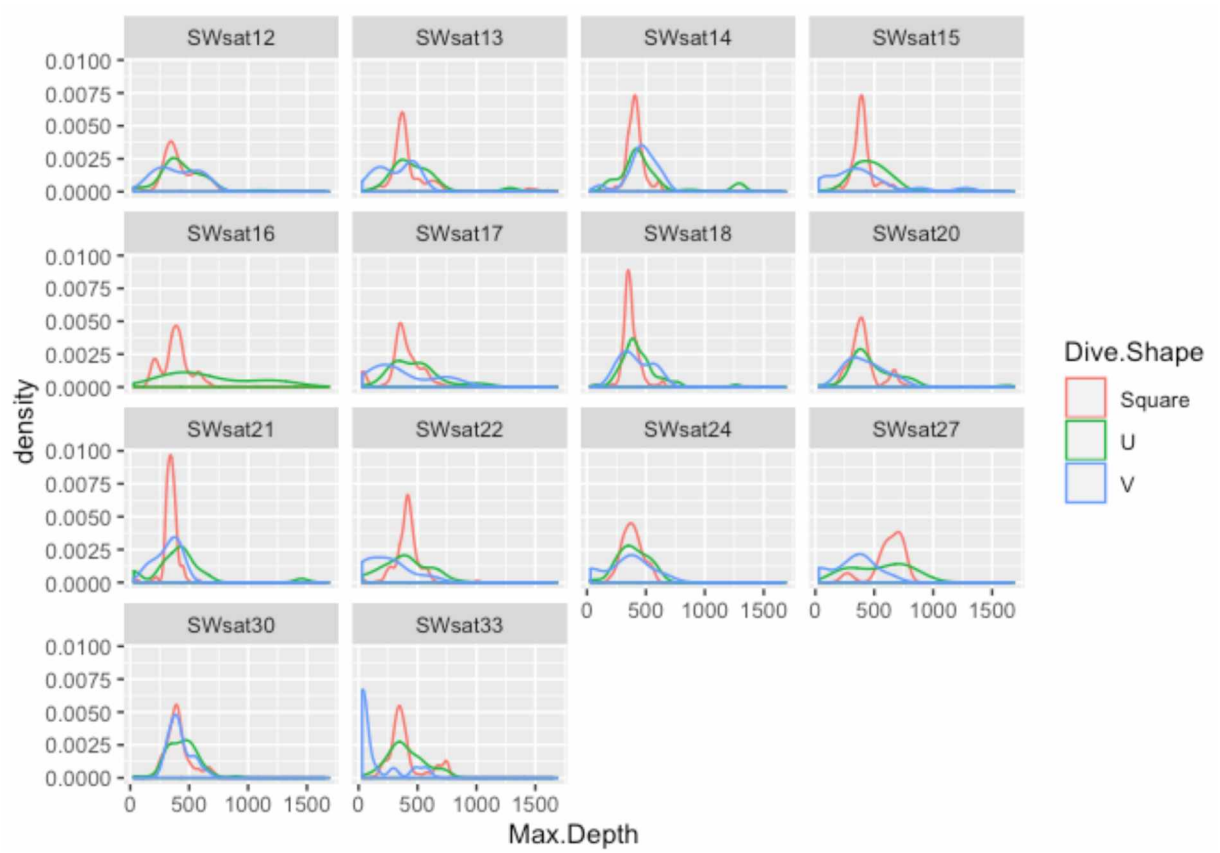

Figure A3.5.2 Maximum dive depth achieved for each dive shape for each individual tagged whale.

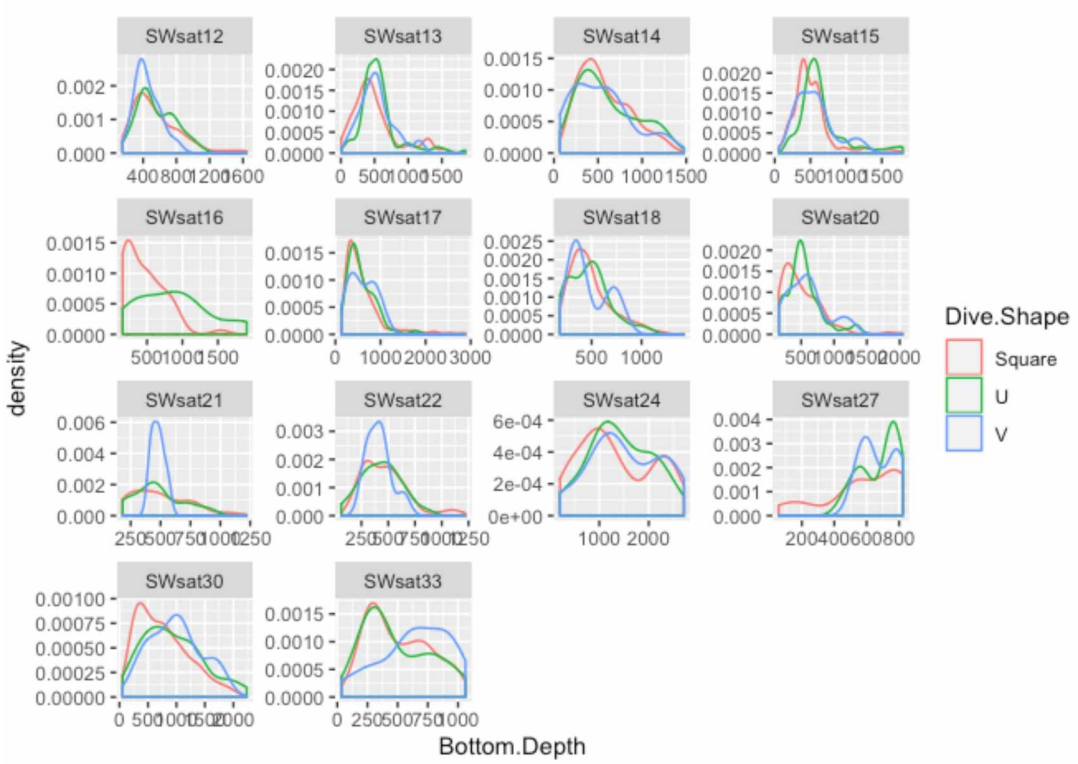

Figure A3.5.3 Seafloor depth at associated dive shape for each individual tagged whale.

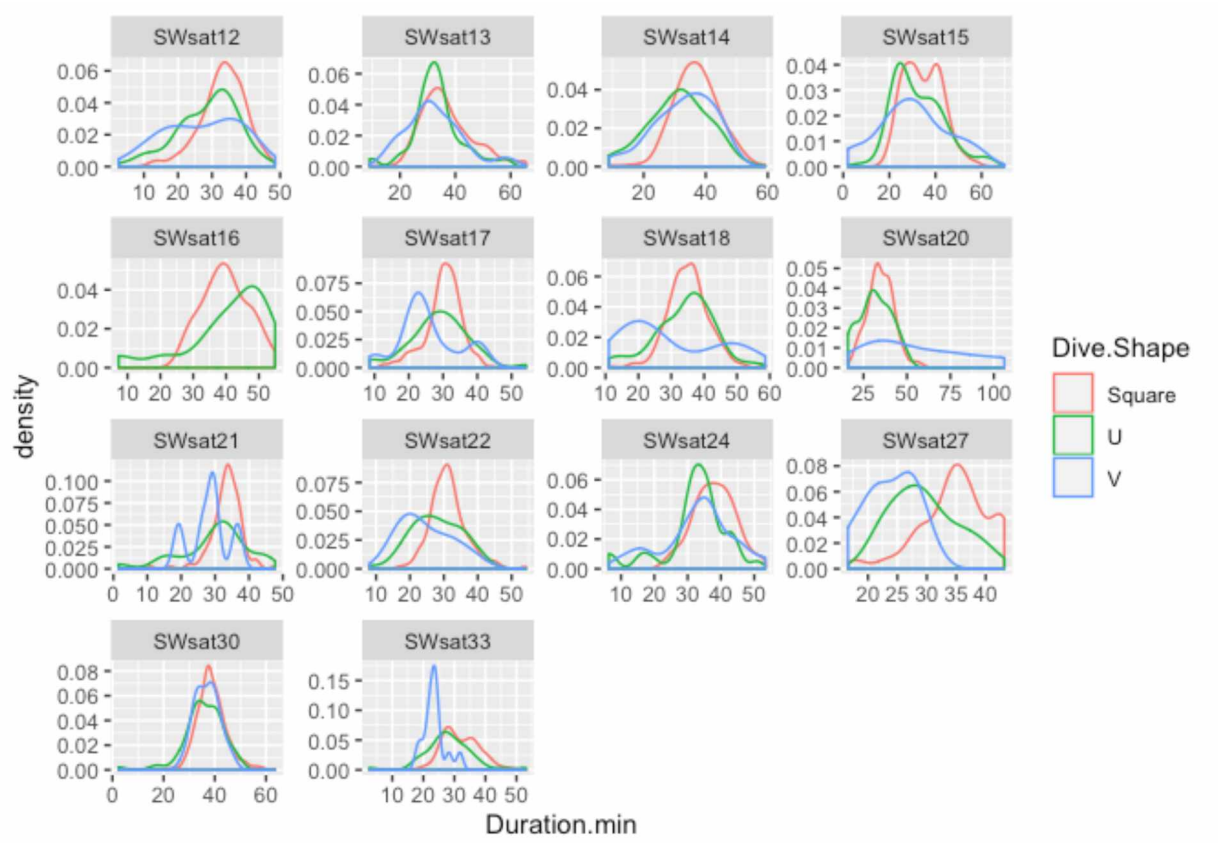

Figure A3.5.4 Dive duration for each dive shape for each individual tagged whale.

Table A3.5.1 Dive shape with respect to light levels.

Light	**# Square (%)**	**# U (%)**	**# V (%)**
Dawn	125 (71)	40 (23)	8 (5)
Day	2164 (77)	543 (19)	111 (4)
Dusk	107 (71)	35 (23)	9 (6)
Night	580 (73)	175 (22)	44 (6)

Table A3.5.2 Dive shape with respect to lunar cycle.

Lunar	**# Square (%)**	**# U (%)**	**# V (%)**
Crescent	579 (74)	163 (21)	44 (6)
Full	338 (77)	76 (17)	23 (5)
Gibbous	707 (73)	224 (23)	42 (4)
New	415 (82)	77(15)	14 (3)
Quarter	937 (76)	253 (20)	49 (4)

Table A3.5.3 Dive shape with respect to mean maximum dive depth, duration, and seafloor depth.

Dive Shape	**Mean Max Depth (m) (±SD)**	**Mean Duration (min) (±SD)**	**Seafloor Depth (m) (±SD)**
Square	394 (±135)	34 (±7)	586 (±386)
U	422 (±224)	28 (±11)	640 (±398)
V	303 (±207)	28 (±13)	714 (±476)

3.8 Supplemental Data

S3.1 Full tag movement records

Full tag tracks for all whales show five individual tagged whales moved south of Washington state while tags were still transmitting (Figure S3.1.1). Whales generally stayed over the continental shelf edge while in the GOA and while migrating south (Figure S3.1.1), rather than moving out to the deep ocean basin. No whales migrated toward Hawaii or into the central North Pacific (Figure S3.1.1).

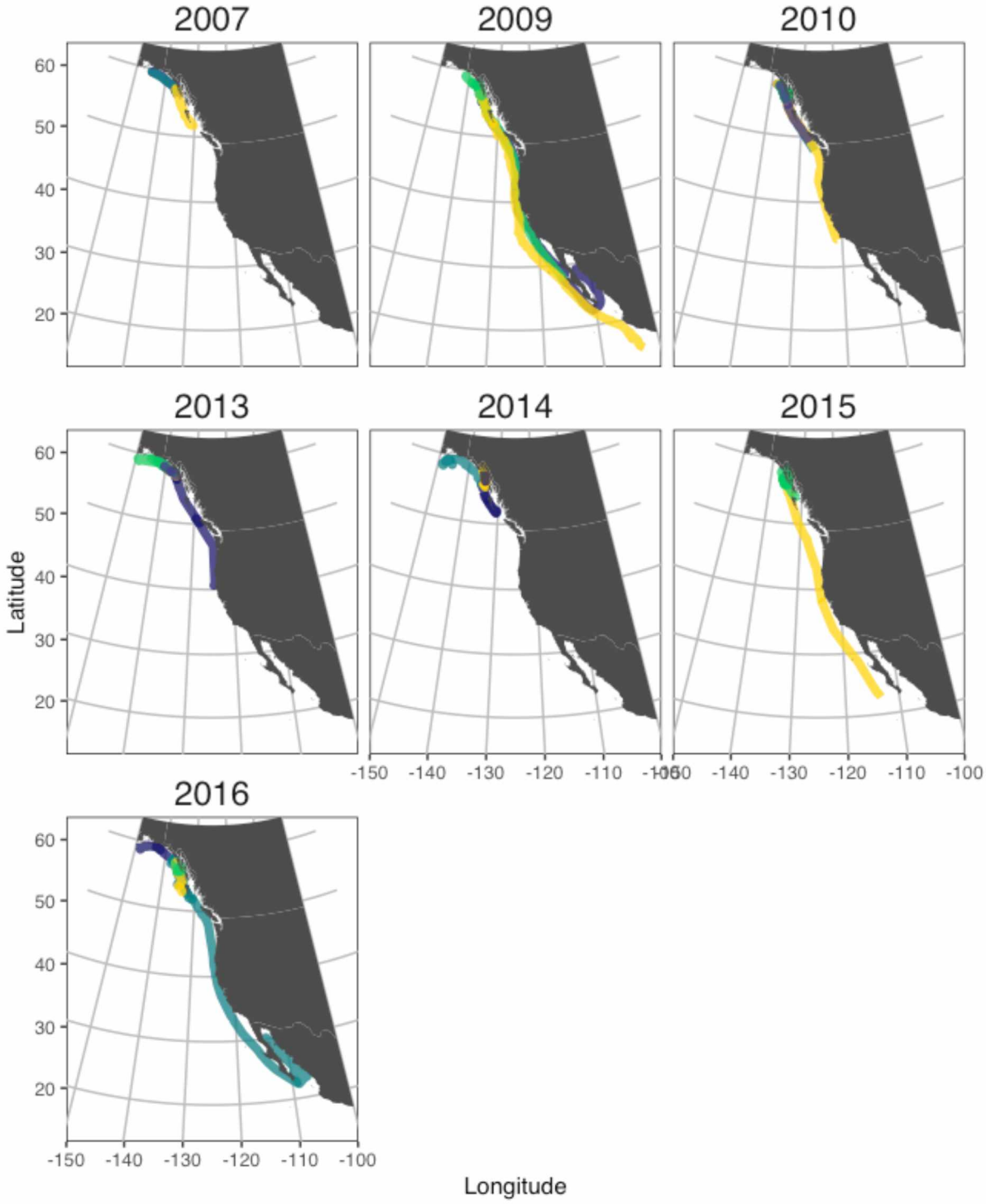

Figure S3.1.1 Full tag tracks for all 29 tags analyzed for this study. Five tagged whales moved south of Washington state while tags were still transmitting; two in 2009, one in 2010, one in 2015, and one in 2016.

S3.2 Time series data

Dive depth data were collected at low resolutions in 2min 30sec increments on days when a tag was programmed to transmit, providing a rough estimate of dive depth data throughout these time periods (Figure S3.2.1). By zooming in to specific days or 24-hr periods, we were able to explore the variability in maximum dive depth over time and identify shifts between shallower and deeper dives (Figures S3.2.2 & S3.2.3). At some periods it appears whales are tracking the bathymetry (e.g. Figure 3.S2.3, bottom panel, September 23 from roughly 11am to 1pm); at others, the dive depth appears to oscillate back and forth between two similar dive depths (e.g. Figure S3.2.3, top panel, May 21 from roughly 7am to 12pm).

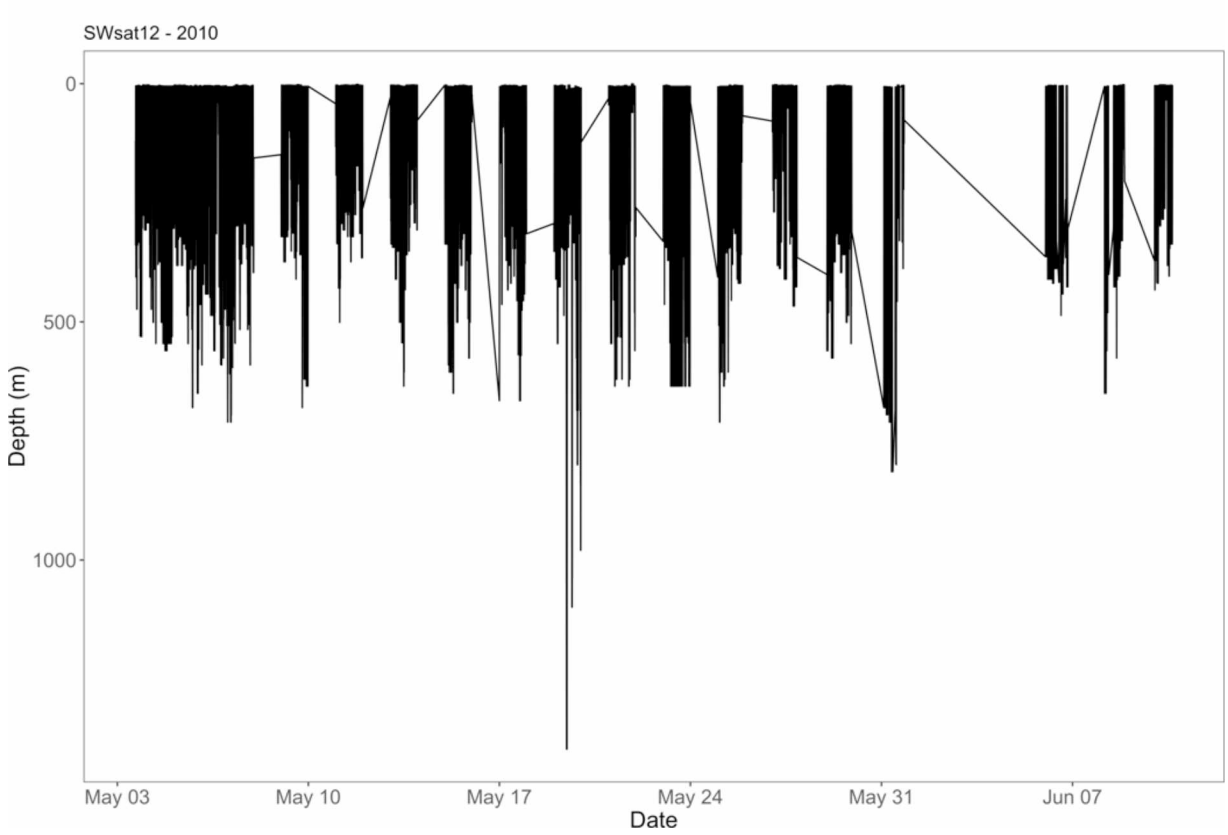

Figure S3.2.1 Time series data show dive depth data collected every 2min 30 seconds from SWsat12 in 2010. The tag collected time series data for the first 5 days the tag was on, and then went to a duty cycle of one day off and one day on. The break between June 1 and June 7 was likely due to a failure of the tag to transmit a full time series message on the "on" days.

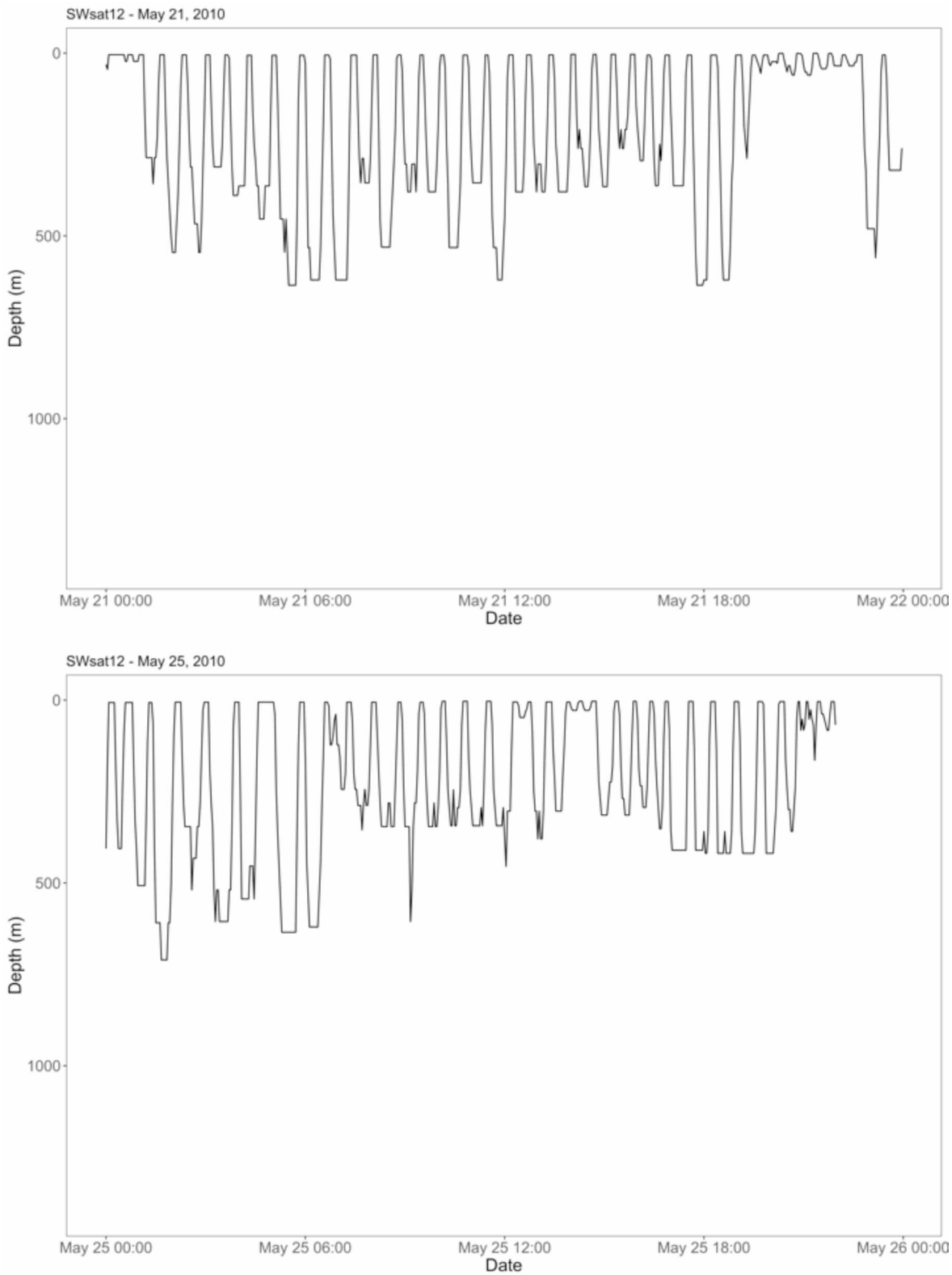

Figure S3.2.2 Dive profiles from tag time series depth data for SWsat12 on May 21st (top) and May 25th (bottom) in 2010 showing individual dives.

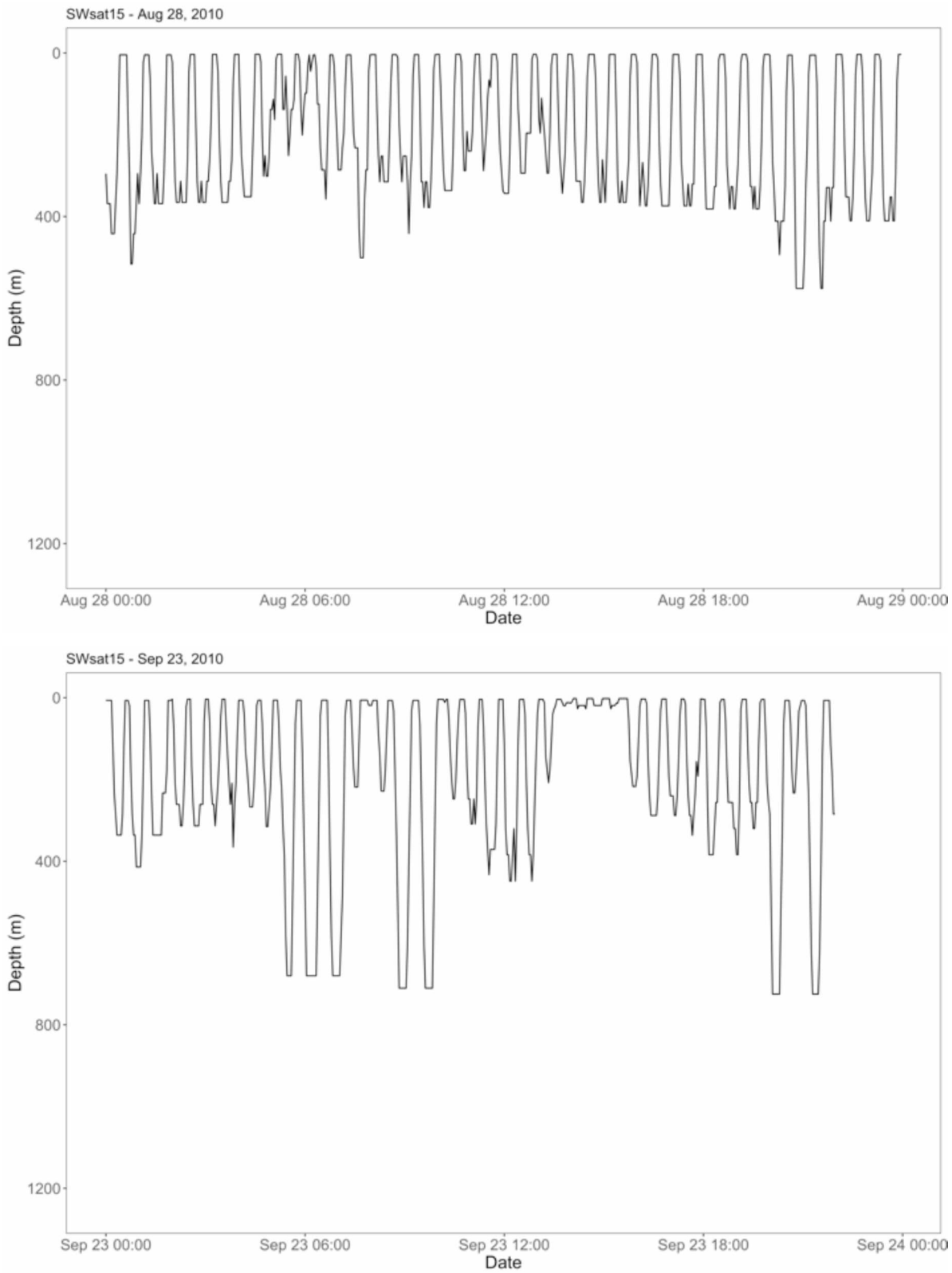

Figure S3.2.3 Dive profiles from tag time series depth data for SWsat 15 on August 28th (top) and September 23rd (bottom) in 2010, showing individual dives.

General Conclusions

Sperm whale depredation of sablefish from commercial longline fishing vessels in the Gulf of Alaska provides researchers with a unique opportunity to study this endangered species. Through collaborations with fishermen, the SEASWAP team has used fishing vessels as research platforms to conduct a majority of the research presented in this dissertation. These collaborations have also allowed frank and important conversations to happen between managers, academics, and industry, to formulate important research questions that benefit all parties. This dissertation focused on exploring two important topics in sperm whale life history: diet and movement of males in high latitudes.

Chapter 1 addressed uncertainty in the time period reflected in the stable isotope signature of cetacean skin. Pieces of humpback, fin, and sperm whale skin were subset into three layers and three cores to explore isotopic variability within the skin. Results showed isotopic variability was greater among layers than among cores of skin, providing evidence of dietary time series among layers of cetacean skin. The humpback whale skin had higher $\delta^{15}N$ values (representing trophic position) in the outer layers of skin, which likely reflected foraging on herring in the spring, while the inner layer of skin had much lower $\delta^{15}N$ values, likely reflecting feeding on euphausiids in the fall. Meanwhile, the fin whale skin showed low variability likely due to their fairly homogenous year-round diet of euphausiids. The sperm whale skin showed significant differences in $\delta^{13}C$ values, and when the sample size for sperm whale samples was increased, stable isotope ratios were found to vary significantly with respect to layer and day of the year (DOY). As a metric of diet over time, isolating the inner layer of skin likely provides information on the most recent diet of a whale, while the outer layer of skin likely represents the oldest dietary information of whales.

Our findings inform scientists conducting dietary research on cetaceans using stable isotopes, in that the portion of skin used may be important for some research questions. For studies focused on diet in species where foraging preference may change over time or the species may migrate, the inner layer of skin is more temporally matched to recent dietary information, making it better to use in prey comparison studies or with isotopic mixing models to assess prey contribution to overall diet. However, for studies focusing on general isotopic niche and trophic level calculations, a full-thickness homogenized sample of skin may provide a more appropriate

long-term average of diet composition. Further research with cetacean skin should seek to expand on this research to examine specifically how stable isotopes move through the skin tissues and at what temporal rates. Additionally, a better understanding of how quickly after an animal changes its diet does the isotope ratio begin to change in the skin, and how quickly the dietary signature of that prey moves through the different layers of skin, are useful further questions on this topic. Overall, our study has found that it is imperative that researchers communicate the portion of skin used in isotopic studies to allow the scientific community to better assess conclusions taken from isotope studies.

Chapter 2 addressed questions from commercial longline fishermen in Southeast Alaska regarding the diet of depredating male sperm whales. Fishermen wanted to know how important sablefish were to sperm whales historically, and what proportion of their overall diet is made up of sablefish today. Historical whaling records indicate sperm whales in the GOA generally ate ragfish, rockfish, dogfish, skates, and squid. Sablefish did not appear to contribute to diets in the GOA and offshore of northern British Columbia, but did appear in stomach contents of sperm whales killed further south along the US West Coast, in the California Current. In general, our mixing model results for current feeding patterns suggest that sperm whales sampled in the eastern GOA primarily consume sablefish, dogfish, skates, and rockfish. Due to similarities in bulk isotope signatures of sablefish and dogfish, we had to combine those two species for mixing models. The prevalence of skates, rockfish, and dogfish is consistent with historical stomach contents data, but the notable lack of ragfish and the addition of sablefish distinguishes more recent diets. Whales sampled between 2003 and 2009 had a smaller proportion of sablefish/dogfish in their diets than whales sampled between 2010-2017, indicating a potential increase in sablefish consumption over time. This complements fishermen's observations that depredation has been increasing over time. It also aligns with managers findings that in the eastern GOA region (EGOA) depredation rates have increased since the mid-1990s (Hanselman *et al.*, 2018a). Sperm whales also appear to increase sablefish/dogfish consumption throughout the summer fishing season. The most surprising results were that the individual whales from our sample that were sighted most frequently around fishing vessels appear to prefer skates to any other prey. This finding could be attributed to a combination of small sample size, a few individuals driving the preference, and timing of sampling for these individuals.

In general, chapter 2 represents an important first step in describing foraging ecology of sperm whales and their prey in the GOA using stable isotopes. The lack of sablefish as sperm whale diet in historical stomach contents records from commercial whaling suggests that sablefish is a relatively new diet item for sperm whales, and depredation reflects a new source of mortality that sablefish did not experience in the past. Sablefish currently make up over 50% of the diet for sperm whales in this region, which is due at least in part to depredation activity. We suggest that sperm whales have not only changed behaviors to target sablefish but may have actually switched prey in response to discovering the easy availability of the lipid-rich sablefish food source. Future work to compare the energy content of all prey items in this study may inform whether or not whales have switched to a better prey resource in sablefish, and more importantly, may speak to ecosystem functions and how changes in trophic pathways may be impacting energy flow and ecosystem functions. While the recovery of whales after the cessation of commercial whaling is often cited as a major contributor in resource conflicts throughout the world, our work shows that, at least for sperm whales, the conflict could simply have arisen from whales adopting a new diet in response to an opportunity, regardless of the changes in the number of sperm whales.

Chapter 3 shifts gears from diet to movement of sperm whales in the GOA and explores movement and diving behavior of depredating male sperm whales through the data analysis of satellite tags deployed between 2007 and 2017. The motivation for this work was twofold. First, little is known about the local movement, broad-scale migrations, and foraging and diving behavior of male sperm whales in high latitudes; this tag data set represents an opportunity to increase scientific knowledge of this species. Second, increased understanding and identification of foraging hotspots can help fishermen avoid fishing near sperm whales at certain times.

We analyzed movement data from 29 satellite tags deployed on male sperm whales in the eastern GOA from 2007 to 2017, 14 of which included dive behavior data. We used state-space models to interpolate hourly positions and estimate behavioral states. Finally, we used generalized additive models (GAMs) to evaluate environmental covariates that influence dive depth and duration. Our results have shown that male sperm whales in the GOA migrate over long distances, staying over the continental slope habitat, and do not migrate to Hawaiian waters or other regions across the deep ocean basin. Minimum rates of horizontal movement were lower in the GOA, and whales sped up when they left the GOA heading south. Additionally, behavioral

states changed when whales left the GOA heading south, from a majority of locations classified as foraging to a majority of locations classified as transiting. We showed that some individual sperm whales use the inside waters of Chatham Strait and Lynn Canal in the fall, and that while sperm whales tagged in the eastern GOA move both north and south, more move south. None of the tagged whales ventured west of 148°N latitude while tags transmitted. Migration timing was unpredictable: sperm whales tagged in the GOA between May and September departed Alaska to head south in the summer, fall, and winter months (June through January.

Dive behavior analysis in chapter 3 showed that whales in the GOA likely dive to both the mesopelagic zone and the bathypelagic zone. Whales dove deeper and shorter during the daytime, potentially responding to diel shifts in prey, and deeper and longer during quarter moons, when tidal currents are weakest. Dives were deeper as seafloor depth increased, up to about 800 m seafloor depth, before dives became shallower with deeper seafloor depths. This pattern could be due to whales switching from bathypelagic prey to mesopelagic prey when seafloor depths became deeper than 800 m, perhaps due to energy expenditures for deeper dives outweighing energy gains from the bathypelagic prey. As CPUE increased, dive depth decreased, which could be a reflection of depredation behavior during which whales do not need to dive as deep to access fish from fishing gear. Individual variability in dive behavior explained a lot of the variability of both maximum dive depth and dive duration models, where some whales are deep divers, and some whales are longer divers, on average. Overall, chapter 3 represents a first look at satellite tag data from a high latitude male sperm whale population, including timing of migration, range, behavioral state estimation, and dive behavior analysis.

This work also highlights the need and direction for future research. Our stable isotope study provided a first look at diet and trophic connections between sperm whales, groundfish, and squid in the eastern GOA. Increasing sampling effort to attempt to get to the bottom of the lack of ragfish would benefit future studies. Additional research to better understand the isotopic incorporation rate for sperm whales specifically would be useful to understand the period of time reflected by the inner layer of skin. Compound-specific isotopic analyses of essential and non-essential amino acids could offer an increased level of detail and insight into dietary pathways (Popp *et al.*, 2007; Crawford *et al.*, 2008; Newsome *et al.*, 2010). These methods could be used to isolate specific prey sources contributing to sperm whale diets, separate the sablefish and

dogfish prey to improve proportional contribution of each species to overall diets, and discriminate between dietary changes and shifts in nutrient regimes (Crawford *et al.*, 2008).

From a movement perspective, there are specific data sets that could provide additional insight into our findings. First, deploying tags of shorter duration that collect and/or transmit high resolution dive data would benefit dive behavior studies. These data, with no dive threshold cutoff, could help identify an appropriate dive threshold depth for sperm whales, and could perhaps then be used to better identify behavior associated with various dive depths. The ability for satellite tags to transmit larger amounts of data while an animal is at the surface would greatly increase our ability to collect finer scale movement and diving information about these species. Behavioral state space modelling could be improved through a number of methods. The rapidly changing field of SSMs has grown considerably even over the course of this dissertation, with new and faster modelling techniques available. Within four months of writing this dissertation, a faster hierarchical SSM package was introduced that uses template model builder to run models smoothly and efficiently in R (Ian Jonsen, pers comm.). There have also been recent advances in the ability to estimate more than two behavioral states, and to do so by adding additional dimensions to input datasets (McClintock and Michelot, 2018). These newer models could give us the capability to use location and dive data together to help estimate behavioral states, which might improve the state estimation done in this study. Environmental covariates can also be input to these new models, further improving the models' abilities to estimate whether animals are foraging, transiting, resting, or searching for prey (McClintock and Michelot, 2018). At present, they have been tested on some pinniped data, but not with large cetaceans where tags collect data slightly differently between location and dive covariates. Finally, focusing both tagging and biopsy efforts to winter and spring, though difficult logistically, would provide SEASWAP with much-needed information about the diet and movement of these animals during poorly known seasons. These data would fill in gaps in our knowledge about diet variability and movement.

Altogether, the work from this dissertation increases our knowledge of male sperm whale diet and movement in their high latitude foraging grounds in the GOA. For the first part of this study, we used stable isotope analysis as a proxy for diet, to better understand trophic connections and dietary preferences of sperm whales with respect to their prey. Our results from chapter 1, finding that a dietary time series exists in cetacean skin, was used to inform our

selection of tissue to sample for sperm whale diet in chapter 2. Specifically, our finding that the inner layer of skin represents more recent dietary information directed our use of the inner layer of skin from sperm whale samples to isolate more recent dietary information. This allowed us to be more confident that the dietary information we were testing from sperm whales in chapter 2 reflected their diet while they were in Alaskan waters.

Our findings from chapter 2 complement and relate to our results from chapter 3. The fact that satellite tagged sperm whales sped up when they departed the GOA and switched behaviors from primarily foraging (79%) to primarily transiting (69%) indicates that the GOA is an important foraging ground for these whales, which is valuable information for conservation and future recovery of this species. Tagged whales did not move west from the EGOA and West Yakutat (WY) management regions to the central or western GOA management regions. Indeed, NMFS scientists report sperm whale depredation to be more common in the EGOA/WY area than the central or western GOA, yet sablefish biomass estimates are greater in the central GOA than any other management area (Hanselman *et al.*, 2018b). We suspect that whales would likely move to areas of higher sablefish abundance (e.g. CGOA) if sablefish were targeted in natural foraging activities in addition to depredation. Instead, we find that whales tagged in the EGOA region primarily stay in the area for depredation and did not move to the western or central GOA. While this could simply indicate that there are plenty of foraging opportunities in the EGOA area, it could also suggest site fidelity or residency of these whales to this region, and perhaps low importance of sablefish to natural foraging activity, as evidenced by our finding that sablefish were not a main historic diet item. Our finding that the tagged sperm whales in this study preferred the narrow band of the continental slope habitat aligns with our dietary findings of prey preferences for groundfish found primarily in that habitat. Sperm whales tagged in this study preferred the upper continental slope and shelf habitat. This further supports our dietary findings of prey preferences of skates and rockfish in particular, species that both tend to be found in larger biomasses in slightly shallower waters than less preferred diet items such as grenadier.

Tagged whales dove deeper during daytime, perhaps in response to diel cycles of their prey. Our findings from chapter 2 indicate these whales primarily forage on sablefish, dogfish, rockfish, and skates, with sablefish, dogfish, and some skates all exhibiting diel vertical migration (DVM), generally preferring deeper water during the daytime, and rising vertically at

night (Carlson *et al.*, 2014; Peklova *et al.*, 2014; Goetz *et al.*, 2018; Sigler and Echave, 2019). Little is known about the movements of the specific squid species sperm whales eat in the GOA (*B. magister* and *O. robusta*), used in our dietary analysis, but squid are known to perform DVM throughout the world as well (Davis *et al.*, 2007).

Seafloor depth was significantly correlated to maximum dive depth and duration, with dives generally increasing in duration as seafloor depth increased, suggesting increased time spent finding and capturing prey, as well as increased time spent descending and ascending from dives. The maximum depth of dives had an increasing and then decreasing relationship with seafloor depth, suggesting prey switching or responses to energy expenditures during deep dives. Our diet analysis suggests sablefish, dogfish, rockfish, and skates are the primary prey of sperm whales in this region, with some magister squid and robust clubhook squid as well. These important prey species inhabit a range of depths along the continental slope; sablefish tend to inhabit waters between 326-553 m but have been found to occur in depths up to 1600 m (Sigler and Echave, 2019); spiny dogfish inhabit waters of the upper slope and shelf regions in depths up to 675 m, and are distributed throughout the water column (Castro, 1983; Tribuzio *et al.*, 2018); rockfish tend to be distributed in rocky slope and shelf habitats, occurring in waters depths generally between 300-600 m, but little is known about their vertical movements (Tokranov and Davydov, 1997; Clausen and Fujioka, 2007; Echave and Hulson, 2018); and skates are wide-ranging, with many of the species found in continental slope waters (*Bathyraja* spp.) occurring in depths over 1000 m, but most abundant in waters up to 200 m (Ormseth, 2017). Given our maximum dive depth results, whales may potentially be targeting many of these groundfish species in water depths less than 800 m where they are more abundant. In deeper water, where seafloor depth exceeds 800 m and preferred groundfish prey becomes scarcer, whales may be switching to mesopelagic prey, which don't require them to dive to the seafloor. This may account for some of the squid found in their diets, or perhaps some dogfish distributed off the seafloor. Our results also showed that sometimes whales will continue to dive to increasing depths as the seafloor depth increases past 800 m, which may reflect continued foraging on species such as skates and sablefish that inhabit deeper water, and which comprised a large proportion of diets. Large squid such as *O. robusta* are poorly understood in the North Pacific, but are thought to exhibit DVM, and may be targeted by whales over deeper seafloor depths.

Marine mammals and fisheries interactions are only going to increase as marine mammal populations recover from commercial whaling and our fisheries grow. Marine ecosystems are an intricate web of interactions that are suspended in a delicate balance; humans' place in this ecosystem requires conscious acknowledgement and respect of our powerful influence and ability to have a profound impact on the balance of marine systems. In Alaska, fisheries management has largely shown success stories, but for fisheries like the sablefish fishery, the future of managing our fisheries resources will depend heavily on how we balance and manage marine mammal interactions. To do this, we must understand how each species fits into the balance, how they interact, and what factors most influence their occurrence and success.

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Appendix A: IACUC Approval

Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

May 13, 2016

To: Jan Straley
Principal Investigator

From: University of Alaska Fairbanks IACUC

Re: [906340-1] Biology of sperm whales in the Gulf of Alaska

The IACUC reviewed and approved the New Project referenced above by Full Committee Review.

Received:	May 4, 2016
Approval Date:	May 12, 2016
Initial Approval Date:	May 12, 2016
Expiration Date:	May 12, 2017

This action is included on the May 12, 2016 IACUC Agenda.

PI responsibilities:

- *Acquire and maintain all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol and could result in revocation of IACUC approval.*
- *Ensure the protocol is up-to-date and submit modifications to the IACUC when necessary (see form 006 "Significant changes requiring IACUC review" in the IRBNet Forms and Templates)*
- *Inform research personnel that only activities described in the approved IACUC protocol can be performed. Ensure personnel have been appropriately trained to perform their duties.*
- *Be aware of status of other packages in IRBNet; this approval only applies to this package and the documents it contains; it does not imply approval for other revisions or renewals you may have submitted to the IACUC previously.*
- *Ensure animal research personnel are aware of the reporting procedures on the following page.*

(The following information is also available in a printable format in the IRBNet Forms and Templates)

HOW DO I REPORT CONCERNS ABOUT ANIMALS IN A UAF RESEARCH FACILITY?

- All "live" animal concerns related to care and use should be reported to the IACUC
- Email: uaf-iacuc@alaska.edu Phone: 474-7800
- Report form: www.uaf.edu/iacuc/report-concerns/
- IACUC Committee Members: www.uaf.edu/iacuc/iacuc-info/
- Additional information: www.uaf.edu/ori/responsible-conduct/research-misconduct/ and www.uaf.edu/ori/responsible-conduct/conflict-of-interest/

WHAT SHOULD I DO IF AN ACCIDENT OR INCIDENT OCCURS IN AN UAF ANIMAL FACILITY?

- **For all immediate *human* emergencies call 911** or UAF Dispatch at 474-7721 for less immediate emergencies.
- If you have **suffered an animal bite or other injury**, complete an "Accident/Incident Investigation form" (personal injury) form available at www.uaf.edu/safety/incidentreport-2012.pdf.

- If an accident such as a **chemical spill** occurs, contact the Environmental Health, Safety, and Risk Management (EHS&RM) Supervisor at 474-5617 or the Hazmat Coordinator at 474-7889.

WHO DO I CONTACT IF I FIND A DEAD, INJURED, OR DISTRESSED ANIMAL IN A UAF RESEARCH FACILITY?

- During regular business hours, immediately contact facility staff and/or Veterinary Services Staff at 474-7020.
- After hours or on weekends, immediately contact facility staff and/or Veterinary Services Staff using the contact numbers posted on the "Emergency Contact Information" in the facility or call UAF Dispatch at 474-7721.
- Contact the IACUC at 474-7800 or uaf-iacuc@alaska.edu if an "Emergency Contact Information" sign is NOT posted in the facility.
- Contact the IACUC if you are not satisfied with the response from Vet Services.

HOW DO I REPORT ANY CONCERNS REGARDING WORK HAZARDS OR ANY GENERAL UNSAFE CONDITIONS?

- Complete an "Unsafe Condition Reporting Program" form, available at the EHS&RM website: www.uaf.edu/safety/unsafe-condition/

WHERE CAN I OBTAIN GENERAL OCCUPATIONAL SAFETY INFORMATION?

www.uaf.edu/iacuc/occupational-health/